Disseminating Innovative solutions for Antibiotic Resistance Management

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D3.3 State of the art report – Summary of DISARM's participatory database

Participants: Wageningen Research, UGent, IfA, ILVO

Authors: Lisa Morgans (IfA), Ilias Chantziaras (UGent), Helena Ferreira (ILVO), Francesca Neijenhuis (WR), Marc B.M. Bracke (WR)

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1 Glossary/abbreviations

AB: Antibiotics

AMR: Antimicrobial resistance

AMU: Antimicrobial use

Anses: The French Agency for Food, Environmental and Occupational Health & Safety

BDCT: Blanket dry cow treatment

CIAs: Critically important antimicrobials

DCT: Dry cow therapy

DISARM: Research project (Disseminating Innovative Solutions for Antibiotic Resistance Management)

ECDC: European Centre for Disease Prevention and Control

EFSA: European Food Safety Agency

EMA: European Medicines Agency

ES: Spain

ESPRUMA: (ES)

Idele: French Livestock Institute

ILVO: Flanders Research Institute for Agriculture, Fisheries and Food

IMA: The Industrial Minerals Association (EU)

INTERPORC: Spanish Inter-professional Agri-Food Organization for White Pork

ITAVI: The French Poultry Institute

NL: The Netherlands

PCU: Population correction unit (unit developed by the EMA to calculate AB use in food-producing animals)

RUMA: Responsible Use of Medicines in Agriculture Alliance (UK)

SCC: Somatic cell count

SDCT: Selective dry cow therapy

UK: United Kingdom

VETresponsable: Uso Responsable de los Medicamentos Veterinarios (Spain)

WHO: World Health Organisation

WR: Wageningen Research (NL)

WUR: Wageningen University and Research (NL)

2 Introduction

The DISARM thematic network has developed a network linking together farmers, veterinarians, advisors, industry members and researchers to codify and promote best practice strategies to reduce antibiotic resistance in intensive and grazing livestock farming. The network focusses on pigs, poultry and the grazing sector (dairy, beef and sheep). There is real benefit in the exchange of innovative approaches. Different sectors can learn from the approaches to livestock health adopted by innovative farmers in other sectors or countries. The overall aim is to reduce antibiotic (AB) resistance, by reducing the need for AB in livestock farming by focussing on disease prevention and prudent use of AB. Best practices are therefore focussed on improving animal health and prevention of disease.

The DISARM Network developed a baseline assessment of state-of-the-art strategies and technologies to reduce antimicrobial use (AMU) and improve animal health on farms, including strategies developed by farmers, industry and researchers. A library of open access information sources has been developed, which can be used by farmers and their advisors to access information on strategies to reduce AMU and subsequently antimicrobial resistance (AMR) (https://disarmproject.eu/search-resources/; see also Bennani et al., 2020). Best practices and innovations have been selected because they reduce the potential development of AMR. The state-of-the-art report and connected database has been used to feed the community of practice (CoP) with best practices. Vice versa, the database has been fed with best practices from farms, industry and research by the community of practice members.

This synopsis report presents the strategies delivered by the consortium partners in the DISARM thematic network. It establishes the baseline State-of-the-Art for AMU and AMR in livestock farming with innovative strategies from farmers and industry as well as the baseline information from research projects at the global, EU and national level on how to reduce AMR in livestock production. The report summarises the separate entries in the database and is mainly meant to explain the structure of the database and create interest in the reader to explore the database further (https://disarmproject.eu/search-resources/).

This final state-of-the-art review and linked database of strategies has been continually updated with feedback from the CoP, multi-actor farm health plan groups and from the events, workshops and collected best practices developed in work package 5 (WP5) of the DISARM project.

3 Approach

In work package 3 of the DISARM project a protocol was developed for the state-of-the-art materials. An easily accessible Excel file was created with the purpose of not only collecting and organizing the material but also with the specific aim to create an easy to use online presentation of the material for interested parties To process information for this state-of-the-art report and uploading of records on the DISARM website, the authors could only use records/publications for which summaries in English had been provided by the project partners, as only these allowed for a quality check. To structure the information in the database, collected material was subdivided into 10 primary categories. These primary categories were divided in subcategories and several sub-subcategories to enable the possibility of a detailed search (Table 1). The structure of the database is presented in Table 2.

Table 1 Structure of the state-of-the-art database in categories.

Category	Subcategory	Sub-subcategory
Biosecurity	External biosecurity	Animals[#] People Materials Vehicles[#] Air Vermin/pest control Cadavers[#]
	Internal biosecurity	Animals[#] People[#] Materials[#] Vehicles Air Vermin/pest control Housing[#] Cadavers
Pathogen management	Vaccination[#]	
	Eradication[#]	
	Managing sick animals[#]	Targeted use of antibiotics[#] Diagnostics[#] Euthanasia[#]
Housing and welfare	Weaning age and management[#]	
	Enrichment[#]	
	New housing systems[#]	
	Climate[#]	
	Stocking density	
	Milk parlour	
	Pasture (management)	
	Manure management[#]	
Water	Water quality[#]	
	Water system	

	Water additives#	
Feed /gut health	Early feeding (colostrum/feed) #	
	Feeding management#	
	Feed composition#	
	Feed additives and supplements	
Precision Livestock Farming & early detection	Sensor technology#	
	Big data analyses#	
Breeding for disease resistance or robustness		
Specific alternatives	New antibiotics#	
	Immunomodulators#	
	Pre-/probiotics#	
	Other#	
Antimicrobial use reduction strategies	Legislation/Incentives#	Government# Chain/labels#
	Monitoring/Surveillance#	Disease/health# Antibiotic use#
Prudent Use	Farmer# Veterinarian# Pharmaceuticals# Agri-advisor#	
Other*		

* When material does not refer to one of the ten categories. # Categories used in the final database

Although it is a specific interest to enhance cross-pollination between sectors, interested parties might want to search for papers or innovations on specific species or countries. Therefore, a species and country indication was provided (using bold face and underlined text respectively). On every entry, additional information was provided to enable readers to get an impression of the material at hand, before diving deeper into the material (via the provided links) themselves. To give an indication of the level of innovation and of the evidence presented in the farm and industry led innovations, an expert judgement was added in the form of a one to five star rating. Table 2 presents the information that is (as far as known) provided on each entry in the database.

Table 2 Information on entries in database

Information	Categories
Species	Pigs Poultry Dairy Beef cattle Sheep Multiple species
Age category	Young Adult

	All
Scientific paper/report*	Scientific paper Report
Funding body*	Public Private (sector) Private (industry) Unknown
Study design*	Review Experimental study Field study Intervention study Questionnaire Descriptive
Level of efficacy	Reducing antimicrobial resistance Reducing antimicrobial usage Improving animal health Reducing risk factors
Animal welfare	Improved Unchanged Decreased Unknown
Practical - implementation	Easy With some effort Difficult Unknown
Practical - requirements	Management change Purchase materials (Re)construction Unknown
Cost benefit - category	Economical (farmer) Social and/or public health Sector Unknown
Cost benefit - result	Positive Unchanged Negative Unknown
Innovation rating**	1-5 stars
Evidence rating**	1-5 stars

* indicated in research papers and reports only
** indicated in farm and industry innovations only

All partners in the DISARM consortium were asked to search for research papers and farm and industry innovations, deriving preferably from their own country and regarding species and housing systems relevant to the project. However, since cross-pollination is an important way of innovating, some entries regarding other species were also included when the strategy or innovation was regarded of interest for other sectors. Partners were asked to collect material published after 2010, to enhance collection of newer strategies and innovations.

Consortium members supplied information and links to the database, and were involved in the reviewing of this state-of-the-art report.

4 Collected material

A total of 522 records entries were collected by the consortium partners. Of these, 511 records were left after internal quality checks (e.g. by removing double entries) for reporting and uploading on the DISARM website. In total the database includes 340 research papers and research reports, 46 farm innovations, 82 tools and checklists and 43 industry innovations. Table 3 shows the number of entries included in the database in the different categories, and Table 4 shows the number of entries per species.

Table 3 Numbers of collected papers and innovations divided over categories. AMU: antimicrobial use.

	Research papers	Tools and checklists	Farm innovations	Industry innovations	Total
Biosecurity	40	5	7	3	55
Pathogen management	45	6	6	4	61
Housing and welfare	16	5	3	3	27
Water	6	2	4	1	13
Feed /gut health	33	1	5	7	46
Precision Livestock Farming & Early detection	11	1	2	7	21
Breeding for resilience	8	0	1	2	11
Specific alternatives	33	0	3	2	38
AMU reduction strategies	79	6	12	10	107
Prudent use	49	16	3	3	71
Other	20	40	0	1	61
Total	340	82	46	43	511

Table 4 Collected material divided over ‘species’-classification

Species	Research papers and reports	Tools and checklists	Farm innovations	Industry innovations	Total
Beef	7	1	0	0	8
Dairy	86	26	12	12	136
Pigs	93	21	7	9	130
Poultry	49	7	19	7	82

Sheep/goats	10	8	1	0	19
Multiple/other	95	19	7	15	136
Total	340	82	46	43	511

5 State-of-the-art strategies

In this chapter a summary of database entries is presented, divided over the aforementioned categories. Further details regarding the strategies and innovations can be found in the database (https://disarmproject.eu/search-resources/). In the sections below reference to database entries are identified by superscripts in green font like this:[1] (i.e. more information can be found in record #1 in the database). References that were not included in the database have been referenced in the final chapter of the report. Below, different **species of farm animals** have been highlighted in **bold**, and countries where studies have been conducted have been marked with underlined fonts. Each section has highlights summarising key points, and most sections have subsections explaining the concepts, how it relates to AMU/AMR, why it is important, and what it interesting or worthwhile knowing about the topic.

5.1. Biosecurity

Biosecurity measures help to prevent the entry and spread of infectious diseases on and between farms, thereby reducing disease incidence and the need for veterinary antibiotic treatments[160] (Dewulf and Van Immerseel, 2019). The sections below discuss first the database entries on external biosecurity and then on internal biosecurity.

5.1.1. External biosecurity

Highlights

- External biosecurity is the prevention of introducing pathogens, that may cause disease, from entering the farm.
- Aspects of external biosecurity include special attention to disease entry via visitors, animals, wildlife, animal products like semen, equipment, materials like bedding, and via the air.
- A hygiene lock may be one of the most important measures you can take to build external biosecurity.
- The BioCheck.UGent is a freely available checklist you can use to assess various aspects of biosecurity including external biosecurity (www.Biocheck.UGent.be).
- Farmers who want to improve are recommended to consult their local veterinarian to discuss the issue.

What is external biosecurity?

External biosecurity aims to prevent the introduction of pathogens onto the farm through, for example, controlling movement of animals and people onto and off farms; controlling wildlife vectors; and quarantining new animals when buying-in livestock. By contrast, internal biosecurity concerns the spread of pathogens within the farm boundaries (Palczynski, 2021).

How does external biosecurity help reduce antimicrobial resistance?

By preventing the entry of pathogens onto the farm, and into the herd or flock, causing infections, external biosecurity reduces disease and thereby the need for treatment and the subsequent risk of developing AMR (antimicrobial resistance). Antibiotics are used to cure infections caused by bacteria. The other main type of infectious disease is caused by viruses. Viral diseases may lead to increased antibiotic (AB) use due to the increased likelihood of predisposition to secondary bacterial infections. The best way to prevent viral disease is through vaccination.

It is also important to distinguish between disinfection and cleaning. Cleaning implies the removal of visible dirt, whereas disinfection involves killing of (invisible) pathogens like bacteria, viruses, and worm eggs. When animals or manure are present, and esp. when animals live in close proximity to their manure, disinfection is an illusion. Disinfection can only be accomplished in empty barns. When animals are present, cleaning mainly involves taking away the manure.

There have been concerns regarding the use of disinfectants in agricultural environments. However, a study conducted in 2019 reported that proper disinfectant use did not seem to promote AB resistance nor reduce *Escherichia coli* disinfectant susceptibility. Nonetheless, please note that "proper use in agricultural environments" can be a real challenge[170].

It is therefore crucial that disinfectants are used sensibly. In a study involving 51 **veal calf** farms which either reduced AB use alone or in combination with cleaning and disinfection, farms with reduced antimicrobial use (AMU) and farms that acted as control farms showed reduced MRSA (multi-resistant *Staphylococcus Aureus*) carriage in veal calves. On other hand, the additional cleaning and disinfection in these farms had no effect, possibly because it resulted in increased MRSA air loads[316].

Why is external biosecurity important?

Prevention, of course, is better than cure[166]. External biosecurity can help prevent disease, reduce the stress associated with disease and decrease the need for AB treatments.

Biosecurity, in combination with other preventive-medicine strategies such as vaccination, is the basis of any animal-disease control program. When prevention fails, (more) curative action will be necessary, which often includes AB use. Improved external biosecurity has been shown to improve production performance[142,160]. In a Facebook survey covering a range of countries, sectors and professions, biosecurity was recognised as one of the most important prerequisites for animal health (Palczynski, 2021).

External biosecurity has also been often recognized as an important part of AMU reduction. A study showed external biosecurity to be among the top 5 most effective (but not among the top 5 most feasible or most economical) measures by 111 **pig** experts from Belgium, Denmark, France, Sweden and Switzerland to reduce AMU[162]. The link between biosecurity, AMU reduction and improved animal welfare has been fairly well established in **pigs and dairy cattle**, and more recently, Diana et al.[449] reported the need for improved biosecurity. Lower AMU was also related to improved welfare on specialised **beef** farms. Another study pointing out the importance of biosecurity reported that *ESBL-E coli* (extended-spectrum beta-lactamase producing *E. coli*) positive **pig** farms less often had improved biosecurity measures such as a hygiene lock or professional pest control[54].

Furthermore, biosecurity is also important to reduce the risk for zoonoses and food poisoning, e.g. *Salmonella* in **pig** production[153].

However, the importance of biosecurity is still not fully recognized. Farmer perceptions were the object of an online questionnaire to 218 **pig**, 279 **cattle** and 61 **poultry** farmers in

Flanders, Belgium. It was found that the term 'biosecurity' was correctly explained by less than 10% of these farmers[159]. Farmers had broadly similar knowledge on disease prevention and biosecurity. Insufficient motivation was the most likely reason for poor implementation of biosecurity measures. Insufficient information on costs and economic benefit was seen as the biggest obstacle to implementing preventive measures. The veterinarian was considered the main source of information. He can raise farmer interest in disease prevention by explaining the subject in more detail[159].

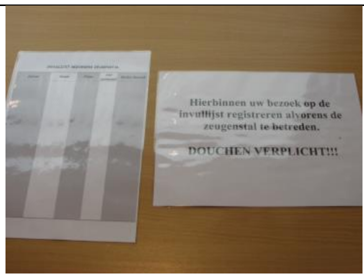
What is interesting & worthwhile knowing about external biosecurity

External biosecurity concerns taking precautions to prevent people, newly purchased farm animals, wildlife/pests, air, equipment or materials (e.g. bedding[37]) to carry pathogens onto the farm. It concerns using e.g. a hygiene lock (including e.g. visitor registration, changing footwear, clothes, taking a shower, disinfection of hands, etc.), imposing a quarantine period for new animals, the purchase of pathogen-free animals, the proper disposal of animals and other materials, and pest and wildlife control.

For example, it has been found that wild animals foraging in the human-influenced environment are colonized by bacteria with clinically important AB resistance[450].

Elements to consider in external biosecurity include the structure of the farm (e.g. a separation of a clean and dirty area), quarantine, purchase of semen and animals; purchase of materials and equipment, disposal of animals and materials; manure; storage of carcasses, supply of feed, water and goods, entrance control, footwear and clothing, a hygiene lock, hand washing, vermin and bird control, farm location and surroundings (items and pictures below derived mostly from De Wulf, J., External biosecurity in pig production. (Presentation). Gent University).

The pictures below illustrate a number of these aspects.

		
Entrance control	Make sure visitors register and know what the rules are	Washing hands

 Taking a shower	 Footwear with profile is more difficult to clean	 Disposal of manure
	 Do not allow unwanted visitors; enforce disinfection	 Pest control: Make sure birds and rodents cannot enter the barn
 A dead mouse	 Make sure rodents do not have a place to hide near buildings	 Disposal of carcasses

Farm innovations mainly regard usage of tools to check the status of biosecurity measures, especially the Biocheck.UGent developed by Ghent University (Belgium, discussed in more detail below), and for **poultry** e.g. PULSE[512,514,515], developed by AIRVOL and ITAVI (France). With such tools farmers can gain insight into aspects of their farm that are well taken care of regarding biosecurity and aspects that require attention to prevent diseases entering or spreading through the farm.

Other industry innovations are e.g. a housing and hygiene concept (HyCare®). The HyCare® system focusses on the hygiene of housing (by using coating of walls and floors), water, cleaning and disinfection and vermin control. Coaching is also included in this concept.

5.1.2. Internal biosecurity

Highlights

- Internal biosecurity refers to the prevention of spread of infectious agents within the farm.
- Internal biosecurity is inherently linked with farm management (disease management, all in / all out (AI/AO), stocking density, compartmentalization and working lines, cleaning and disinfection).
- The BioCheck.UGent™ is a freely available checklist you can use to assess various aspects of biosecurity including internal and external biosecurity (www.Biocheck.UGent.be).
- Farmers who want to improve are recommended to consult their local veterinarian to discuss the issue.

What is internal biosecurity?

Internal biosecurity consists of all measures taken to prevent spread of infectious agents within the farm (e.g. from one age category to another or from one production group to another or even within a production group). Internal biosecurity measures have a very strong link with the farm management and the daily practice of the animal care takers (e.g. hygienic measures between compartments, working lines, cleaning and disinfection practices). In contrast to the external biosecurity measures, these are much more oriented towards the control of endemic infectious diseases.

How does internal biosecurity help reduce antimicrobial resistance?

When experts in **pig** health were asked to rank alternatives to antimicrobial agents based on their perceived effectiveness, feasibility and return on investment, biosecurity ranked first for internal and second for external biosecurity, suggesting that improvements in internal biosecurity are perceived as the most promising alternative to AMU in pig production[7]. Various studies have provided hints regarding the way internal biosecurity can help reduce AMR. For example**, weaner** farms in Denmark that used less antimicrobials than the national median showed a uniform profile with regards to the compartmentalization of the working lines and the use of all in / all out procedures with subsequent cleaning (Fertner et al., 2012). Also in **breeder-finisher pig** herds in Belgium it was found that herds with higher internal biosecurity scores had lower antimicrobial treatment incidences, suggesting that improved biosecurity might help in reducing AMU[160]. In France, farms with distinct working lines and use of all in / all out practices were found to be associated with lower AMU in breeder-finishers herds (Lannou et al., 2012). Last but not least, in a European study involving Belgium, Switzerland, France, Sweden, Denmark and Germany the level of internal biosecurity was found to positively associate with a better control of infectious diseases and a lower need for antimicrobials[128].

Why is internal biosecurity important?

If biosecurity and disease prevention measures are well implemented curative treatment of diseased animals can be restricted to an absolute minimum. Internal biosecurity measures received attention lately due to the intensification of the animal production where animal groups are becoming larger and more vulnerable, and production efficacy is becoming more critical. Also the increasing attention for a reduced and responsible AMU in animal production has promoted the interest in internal biosecurity measures.

What is interesting and worthwhile knowing about internal biosecurity?

The main components of internal biosecurity are:

Disease management

A systematic disease management strategy is needed to protect farm-animal health. It is important to include correct handling and treatment of diseased animals, make use of proper diagnostics, use isolation and disease registration, and ensure a high immunity status for all animals (through vaccination). Diseased animals should be isolated in a sickbay, in order to prevent other animals from pathogen exposure. Any treatment of animals should be performed carefully to avoid mechanical transmission of disease. For example, needles may get contaminated through use and storage by numerous environmental germs and as such become efficient disease transmitters.

All in / All out (AI/AO)

The AI/AO principle helps to prevent cross-contamination between successive production batches and makes it possible to clean and disinfect rooms/units between different batches. A consequence of this principle is that within a batch of young animals (e.g. piglets), any animals that grow slower in comparison to the rest must not be kept in the next batch of younger animals as has been done in the past. Such animals can be a source of infection for the younger age group.

Stocking density

A high stocking density induces stress which results in an increased sensitivity to infections, and an increased excretion of germs. Besides this, decreased animal welfare is associated with high stocking density.

Compartmentalization and working lines

Animals of different age groups may have different levels of sensitivity to certain pathogens and therefore it is crucial to keep age groups separate and to work according to strict working lines starting at the youngest animals, and working towards the oldest animals to end with the quarantine stable and sick bay. To avoid dragging germs on footwear, boot washers and disinfection baths can be placed between production units. For risk-bearing groups (e.g. quarantine stables, sickbay), an additional hygiene lock for changing of clothing, footwear and washing of hands is recommended to avoid pathogen spread between different age groups.

Cleaning and disinfection

To prevent recurring infections between consecutive production rounds, a thorough cleaning and disinfection of pens is required. This consists of the following seven steps: 1) dry cleaning and removal of all organic material, 2) soaking of all surfaces to loosen all remaining organic material, 3) high pressure cleaning with water to remove all dirt, 4) drying of the stable to avoid dilution of the disinfectant (to be applied in the following step), 5) disinfection of the stable to achieve a further reduction of the concentration of germs, 6) rinsing and drying of the stable to assure that animals afterwards cannot come into contact with remaining disinfectant and finally 7) testing of the efficacy of the procedure through sampling of the surface.

The pictures below (courtesy of Prof. Dewulf Jeroen) illustrate these seven steps.

1. dry cleaning and removal of all organic material

2. soaking of all surfaces to loosen all remaining organic material

3. high pressure cleaning with water to remove all dirt

4. drying of the stable to avoid dilution of the disinfectant

Score	KVE per plaat
0	0
1	1-40
2	41-120
3	121-400
4	> 400
4	ontelbaar

5.1.3. Biocheck.UGent™, a tool to check internal and external biosecurity on farms

A substantial number of entries in our database use the Biocheck.UGent™. This is a scoring system developed by the University of Gent to measure and quantify the level of biosecurity on farms. This tool is composed of all relevant components of biosecurity and takes the relative importance of the different biosecurity aspects into account, resulting in a risk-based weighted score.

biocheck .ugent

The Biocheck.UGent™ provides risk-based scores to assess on-farm (external and internal) biosecurity ranging from 0 (worst) to 100 (best). It has been applied on **broiler, pig and cattle** farms.

Risks to external biosecurity for **broiler** farms includes e.g. the purchase of 1-d-old chicks, off-farm movements of live animals, feed and water supply, removal of manure and dead birds, entrance of visitors and personnel, supply of materials, infrastructure and biological vectors and location of the farm. Internal biosecurity concerns e.g. disease management, cleaning and disinfection between batches of animals and taking measures to prevent disease transfer between compartments. Farm data can be filled in for free at www.Biocheck.UGent.be, which serves as a national benchmarking tool.

Preliminary results on 15 **broiler** farms in Belgium showed a huge range in the biosecurity level, with internal biosecurity scores ranging from 54 to 87, and external biosecurity scores ranging from 55 to 72 (max: 100). These first results showed that despite the well-known importance of biosecurity, there is a lack of implementation of many biosecurity measures and room for improvement[150]. Biosecurity was also scored in 399 conventional **broiler** farms in 5 EU member states. Here too, internal biosecurity scored better than external biosecurity. Within external biosecurity, "infrastructure and vectors" had the highest score, while "visitors and staff" had the lowest. Within internal biosecurity, the subcategory "disease management" had the highest score. Internal biosecurity was inversely related to the number of employees and farm size. Results show better education of broiler farmers and their staff may help to improve biosecurity on broiler farms[186].

When the Biocheck.Ugent was applied on 574 **pig** farms in Belgium, Denmark, France, Germany, the Netherlands and Sweden (between 2014-16), it was found that farms in Denmark had higher external biosecurity and less variation between farms (e.g. perhaps because they have more SPF farms)[148].

Irish farrow-to-finish **pig** farms had higher external biosecurity (e.g. purchasing only semen and breeding gilts) and lower internal biosecurity compared to other EU countries[142]. Biosecurity scores explained 8, 23 and 16% of piglet mortality, finisher mortal and ADG (average daily gain) respectively. Thus, lower performing farms need to improve (esp. internal) biosecurity practices[142].

Laanen et al.[160] applied the Biocheck.Ugent on 95 breeder-finisher **pig** herds. The average external biosecurity score was 65 (range, 45–89) and the average internal score: 52 (range, 18–87). Results suggest that biosecurity is generally better implemented in larger herds, in more modern facilities and by younger farmers. External and internal biosecurity scores were positively associated with daily weight gain and negatively associated with feed conversion ratio of fattening pigs. Internal biosecurity scores were negatively associated with disease treatment incidence, suggesting that improved biosecurity may help reduce preventive AMU. This study demonstrates and quantifies a clear link between biosecurity and both production and antimicrobial treatment-related criteria in pig herds[160].

Postma et al.[166] applied the Biocheck.Ugent on 232 farrow-to-finish **pigs** herds in Belgium, France, Germany and Sweden (in 2012-13). Biosecurity on many pig farms was poor and varied between countries. The external biosecurity status was highest in Germany and lowest in France. The internal biosecurity was highest in Sweden and lowest in Belgium. External biosecurity scores were in general higher than internal biosecurity scores. Herds with more sows and more employees were likely to have a higher external biosecurity. A higher external biosecurity positively influenced the number of weaned piglets per sow per year and the internal biosecurity score. A higher biosecurity level, thus, seems to result in healthier animals. These findings promote an improved biosecurity status at pig farms and are of relevance in the discussion on alternative ways to keep animals healthy with a reduced necessity of AMU[166,8].

In a study of 60 German farrow-to- finish **pig** herds it was observed that a low score for external biosecurity and bigger farms (more sows) were associated with a higher AMU in pigs from birth till slaughter. Herds with a higher treatment incidence in growing pigs: i) were located in a region with a high pig density ii) had less strict control for visitors and personnel and iii) had lower 'cleaning and disinfection' scores (internal biosecurity)[176].

Chantziaras et al. (2020) applied the Biocheck.UGent tool for fattening **pigs** in 4 countries (Belgium, Poland, UK and Finland) and found poor external biosecurity scores for farm location and environment in countries with reported outbreaks of ASF (African Swine Fever).

A study involving 30 **pig** and 30 **poultry** farms with a relatively high AMU in the Belgian–Dutch border region showed that more biosecurity measures were implemented on Dutch farms, compared to Belgian farms in both species. In addition, more opportunities were found to increase the level of internal biosecurity compared to external biosecurity in both countries[423].

In **cattle**, Damiaans et al. (2020) applied the Biocheck.UGent tool as a survey on 20 **veal**, 50 **beef** and 50 **dairy** farms in Belgium, after weighing of questions and (sub-)categories based on input from veterinary experts. For all systems, both internal and external biosecurity were low (<50 points out of a maximum of 100 points). Internal biosecurity was generally lower than external biosecurity. Veal farms scored significantly lower for "purchase" than beef and dairy, while scoring higher for the other subcategories of external biosecurity. In dairy and beef, "purchase and reproduction" was the highest scoring subcategory. With this tool, implementation of biosecurity on cattle farms can be assessed in a standardized and reproducible manner. This evaluation allows for benchmarking of farms and herd-specific advice.

5.1.4. Other findings by country and species

Focus on external biosecurity

An intervention study in 70 farrow-to-finish **pig** farms in Belgium, France, Germany and Sweden showed that substantial AMU reduction was possible without negative impact on the technical performance and an overall positive effect on net farm profit[128].

A study comparing 48 intervention farrow-to-finish **pig** farms in Flanders to 69 control farms showed that a substantial reduction in AMU was possible without a negative effect on technical parameters and a positive effect on farm profit, i.e. biosecurity is a cost-effective way to reduce AMU[177].

A study among 227 farrow-to-finish **pig** farms in Belgium, France, Germany and Sweden found that the 44 top-farms (compared to the 'regular' farms) had fewer gastrointestinal symptoms in suckling pigs and fewer respiratory symptoms in fatteners, which could partly explain their reduced AMU and higher performance. They also had higher biosecurity and were located in sparsely populated pig areas. However, 14 of these top farms were located in densely-populated pig areas, but they had higher internal biosecurity and more extensive vaccination against respiratory pathogens. These results illustrate, again, that it is possible to control infectious diseases with low AMU[134].

In the same population of 227 farrow-to-finish **pig** herds in Belgium, France, Germany and Sweden a better external biosecurity was related to a lower AMU from birth until slaughter[167].

Nöremark et al.[122] conducted a survey of visitors on 482 Swedish **cloven-hoofed livestock** farms with reference to the spread of animal diseases. Farms were visited on average 0.3-0.8 times per day, esp. in summer and in small mixed farms. Professional visitors seemed to increase with increasing herd size. Vets, AI-technicians, animal transporters and neighbours often had direct contact with animals or entered housing, and 8.8% of repairmen were also in direct contact with animals. The number of visitors that may spread diseases between farms was associated with animal species and herd size[122].

Simon-Griffé et al.[211] surveyed 100 Spanish **pig** farmers and vets on the current biosecurity measures they were taking. Farmers awarded significantly higher scores to their farms' level of biosecurity than the veterinarians servicing these farms. According to both, the most important biosecurity measures were aimed at minimising the risk of disease introduction by visits and vehicles. Measures to reduce the risk of disease introduction by breeding stock were not applied in many farms. Medium-sized to large farms located in high pig-density regions reported higher biosecurity measures than small herds located in low pig-density areas[211].

Frössling et al.[216] point out that herds that purchase many live animals or have a large contact network due to trade are at a high biosecurity risk. The authors developed a new method to assess disease risk taking direction, temporal order, and also movement size and probability of disease into account. The method may be useful for risk-based surveillance, in the identification of high-risk herds in control programmes or to represent influential contacts in risk factor studies. Risk assessment has been identified as a tool (for vet advisors) for improving external biosecurity at farm level. It was developed in Sweden for **cattle** and **pigs**. The most important factors affecting the risk and the effect of biosecurity measures, such as quarantine protocols and protective clothing, were the frequency of between-farm contacts and prevalence of the disease[220]. A Swedish survey of 368 professionals (vets, AI inseminators, livestock hauliers, animal-welfare inspectors, cattle hoof trimmers) of **pig**, **sheep**, **goat** and **horse** farms found many obstacles to basics like soap and water. E.g. 66% of vets perceived obstacles[136]. Responsibility for biosecurity of both visitors and farmers is key. Farmers must also provide adequate conditions for practicing good biosecurity[136].

A network analysis was conducted of **cattle** and **pig** movements in Sweden[218]. The networks were analysed as monthly and yearly networks, separately per species and with the two species together. The cattle network and the combined network showed a recurring seasonal pattern, while this was not seen in the pig network. Overall, the ingoing infection chain could be a useful measure when setting up strategies for disease control and for risk based surveillance as it identifies holdings with many contacts through live animal movements and thus at potentially higher risk for introduction of contagious diseases.

In another Swedish study, it was found that a highly contagious disease might spread over a large area in the time span of one incubation period. The difficulties in contacting some professionals visiting farms could be a problem in an outbreak situation[219].

Sayers et al.[222] found that farmers in the most **dairy** cattle dense region (in their study) were three times more likely to quarantine purchased stock than were their equivalents in regions where dairy production was less intense. Younger farmers in general were over twice as likely as middle-aged farmers to implement biosecurity guidelines. The owners of large enterprises were almost five times more likely to join a voluntary animal health scheme, and were over three times more likely to pay a premium price for health-accredited animals than were those farming small holdings[222].

Resistant bacteria can persist and spread within and between premises despite declining or zero AMU. Certain aspects of biosecurity repeatedly identified as risk factors for the presence of antimicrobial resistance (AMR) on farms[322].

Bacterial counts on the swab samples from 12 **broiler** houses on 5 farms showed that cleaning that was preceded by an overnight soaking with water reduced bacterial counts more than cleaning without being preceded by an overnight soaking[327]. Moreover, soaking reduces water consumption and working time during high pressure cleaning. No differences were found between protocols using cold or warm water during cleaning. Drinking cups, drain holes, and floor cracks were identified as critical locations for cleaning and disinfection in broiler houses[327].

Sweden has cut AB use on **dairy** farms in four ways: Reducing unnecessary use of AB, minimizing the need for AB and preventing the spread of disease, optimising AB use when needed, and monitoring use and resistance[393].

Risk factors for AB use on 70 **foie-gras poultry** breeding lots in France showed low AB use (31% of batches received at least 1 treatment, and AB treatment frequency index was < 0.3) despite the presence of many risky practices such as having at least one other poultry farm in the vicinity in 60% of cases, multi-ages on the same site in 75% of farms, multi-species farming in 1 out of 10 cases and low use of sanitary huts[265].

Focus on internal biosecurity

In Italy[24], a study focused on the development of a flaming machine for the disinfection of **poultry** grow-out facilities. The trials were run in controlled conditions in the laboratory of the University of Pisa, Italy, and on a private farm. The results obtained were very promising. Test bench trials showed a substantial reduction in *E. coli*, and microbial determinations carried out on-farm did not show any difference between thermal and chemical treatment. Flame disinfection of poultry grow-out facilities could represent a valid alternative to chemical disinfection.

A Belgian study[170] examined the use of disinfectants in **poultry** and **pig** husbandry, and its contribution to the antibiotic and disinfectant susceptibility of *E. coli* strains obtained after cleaning and disinfection. This study showed a high resistance prevalence (> 50%) for ampicillin, sulfamethoxazole, trimethoprim and tetracycline for both species, while for ciprofloxacin only a high resistance prevalence was found in broiler houses. Disinfectant susceptibility results were homogenously distributed within a very small concentration range. All *E. coli* strains were susceptible to in-use concentrations of formaldehyde, benzalkoniumchloride and a formulation of peracetic acid and hydrogen peroxide, indicating that the practical use of disinfectants did not select for disinfectant resistance. No indications for the selection of antibiotic (AB) resistant bacteria through the use of disinfectants in agricultural environments were shown. This study suggests that proper use of disinfectants in agricultural environments does not promote AB resistance nor reduce *E. coli* disinfectant susceptibility.

Another Belgian study[333] focused on the effect of **sow** washing as performed on the farm, on *livestock-associated methicillin-resistant Staphylococcus aureus* (LA-MRSA) skin status and strain diversity. More specifically, washing sows on four MRSA positive pig farms had no significant effect on the MRSA status of the sow's skin or nasal cavities. In 64% of cases, the same strain was detected before and after washing.

In Romania[203], the efficacy of some organic acids (citric acid, malic acid and Adabline ALK product), on bacterial cells and those present in biofilms was tested on 3 bacterial species from the group of Gram negative bacteria (*Esch. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*) and a Gram-positive one (*Staphylococcus aureus*). All organic acids (concentrations of 1% and 2% for each) eliminated the bacterial populations of *P. aeruginosa* and *S. aureus*. The Adabline ALK product for the 2% solution concentration was shown to be more active against *E. coli* and *K. pneumoniae* compared to malic acid and citric acid.

In the Netherlands, Dorado-García *et al.* made a study to evaluate strategies to curb A-MRSA[316]. Fifty-one **veal calf** farms were assigned to one of 3 types of study farm: intervened farms reducing AMU according to protocol; intervened farms reducing AMU according to protocol and applying a cleaning and disinfection program; and control farms without any interventions. MRSA carriage was tested in week 0 and week 12 of 2 consecutive production cycles in farmers, family members and veal calves. This intervention study showed that lower levels of AMU significantly reduced the probability for MRSA carriage in veal calves. The specific cleaning and disinfection program used in this study was not successful, possibly because it resulted in increased MRSA air loads.

A scenario-based workshop with stakeholders was organized by the Agri-Food and Biosciences Institute (AFBI)-NI in December 2015 in Northern Ireland[221] to identify key actors in driving behavioural change in relation to on-farm biosecurity. The discussion showed that training in biosecurity for farmers is important and necessary. Training was recommended to be provided by veterinary surgeons, preferably via a face-to-face format.

The discussion addressing disease disclosure proved particularly challenging between those who were prospective buyers of **cattle**, and those who sold cattle. This workshop provided a unique and invaluable insight into key issues regarding farm-level biosecurity activities.

Another qualitative research study was set up in the UK[242] to help further understand why **dairy** farmers do not engage in disease prevention and control strategies (biosecurity). Using semi-structured interviews informed by a health-psychology approach with 25 dairy farmers, a number of barriers, such as disease testing inaccuracies, types of disease transmission, perceived lack of risk and effectiveness of measures, were identified. Motivators included being advised to undertake measures by veterinarians, and the increased threat and severity of the disease in focus. These results suggest there is an advantage to farm advisors and herd-health professionals understanding and working with the beliefs of individual dairy farmers to target appropriate communication and advice strategies relating to biosecurity recommendations.

Methods of information transfer

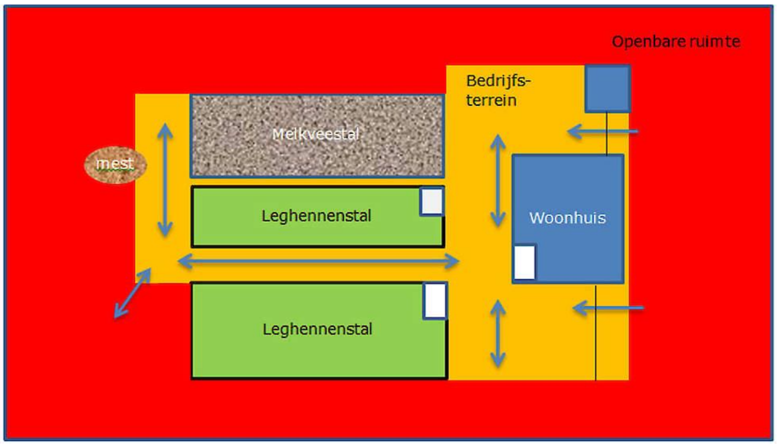
Biosecurity may best be implemented using an argument-based education route (e.g. using on-farm study group meetings with a professional and tools like checklists and software apps). In addition implicit persuasion may be used for promoting single management practices and less complicated messages[81].

Surveys among **pig** and **dairy** farmers and advisors indicate little (appreciation for) use of initial and academic education, and a strong need for professional training and peer exchange of practices and specialised knowledge from animal-health experts[494].

Factsheets, practice guides, information booklets and meetings

Several factsheets and best-practice guides dealing with external and internal biosecurity have been produced, e.g. the ERPA factsheet for rural **poultry** farmers[351], a series of French factsheets for various types of **poultry** farming related to avian influenza (AI)[352,32], a best-practice guide on AMR spread from **poultry** to humans[402], a series of Irish leaflets including one on bioexclusion and biocontainment[404]. This Irish leaflet distinguishes two types of biosecurity practices: 1. Actions taken to reduce the risk of infectious disease coming into your farm, called bioexclusion, and 2. Actions taken to reduce spread of infectious diseases within your farm, called biocontainment. As herds expand farmers need to be even more conscious of implementing bioexclusion practices[404].

The PartAge project[22] concerns biosecurity in conventional and outdoor **poultry** using participatory meetings in France. Bokma et al. (2016) wrote a report (in Dutch) on how **poultry** farmers could deal with AI. One aspect was to use different colours for different parts of the farm premises indicating different levels of potential contamination. Dutch poultry farmers can find information and do a hygiene scan via the Avined website (Avined, No date).



Example of farm areas divided in different colours (taken from Bokma et al., 2016).

An information booklet on reducing lameness in **sheep**[422] addresses external biosecurity in that tackling lameness in sheep requires a number of measures including quarantining all incomers. It addresses also internal biosecurity by emphasizing the need to reduce disease challenges, i.e. when treating lame sheep in the flock, whenever possible segregate out the lame animals to prevent spread between the sheep and keep them separate until fully recovered.

A best-practice guide for intensive **pig** farming is available in Spanish[430], and there are Covid guidelines for **pig** farms in Spanish[431], water management guidelines for **pigs** in Spanish[432], and several plans on AMR[451,452,464], a guide on AB use[453], a booklet describing principles to reduce AB through prevention etc.[454], a number of information sheets[455,456,457,458], a posters/information sheets, e.g. on African Swine Fever, *Salmonella* and biosecurity[459,460,461,462,468], a document on biosecurity from the Ministry of Agriculture, Fisheries and Food of Spain[463], and various information sheets by VETresponsable in Spanish for **pigs**, **poultry** and **cattle**[465,466,467].

A biosecurity manual for healthy **calves** was published by **Dairy** Australia[474], and there is a French guide on lameness in **cattle**[475]. A UK factsheet on Bovine Viral Diarrhoea[173] points out that BVD is an economically important and highly contagious viral disease of **cattle** requiring special attention to persistently-infected (PI) animals. England has an industry-led voluntary scheme to eradicate BVD called BVDFree[173].

A checklist and factsheet was produced on biosecurity practices for **dairy** operations in Texas stating e.g. that not all biosecurity practices will be feasible or necessary for every operation such that individual producers must assess their own risks when deciding which biosecurity practices to adopt[140]. Disease Risk Assessment includes e.g. determining which diseases are the greatest risk to the operation, the cost-benefit ratio of biosecurity for the operation, and how the transmission or introduction of disease on the premises could occur[140].

5.2. Pathogen management

Highlights

- Monitoring and diagnosing causative pathogens is essential when preventing and controlling disease in livestock production.
- Vaccination programmes are useful tools when reducing the impact of disease on livestock health.
- Mastitis and lameness are the major health concerns for **cattle and sheep** and therefore the main reasons for antibiotic (AB) use.
- Diarrhoea and respiratory disease are the major diseases in **pig** production and are often multifactorial.
- Young animals would benefit from improved immunoglobulin transfer to give them the best chance of fighting pathogens.

What is pathogen management?

The term 'pathogen' encompasses a wide range of organisms that are usually harmful or cause diseases. This includes bacteria, viruses, protozoa, nematodes, insects (such as mites and ticks), and fungi. By managing the occurrence and spread of these pathogens, we can limit the damage they cause and the extent of infections and diseases in animals. Pathogen management classically covers the following: prompt identification of the causative agent, i.e. diagnosis, which can be at the animal level or population level in large-scale routine surveillance programmes; various treatment options to include antibiotic (AB) therapy; prevention of a repeated occurrence or prevention of the spread of the pathogen within a population; managing the risk factors that might lead to it occurring or spreading; and the control and eradication of a disease caused by a pathogen through population-level testing and vaccination programmes.

Why is pathogen management relevant to reducing AMU and the DISARM network?

Several bacteria are major disease-causing pathogens that can be treated using ABs. However, bacteria are becoming increasingly resistant to ABs resulting in treatment failures, particularly in human health care settings. If we can reduce the spread and occurrence of the disease from bacterial infections, and this includes secondary infections following viral infection, we will be less reliant on AMU for curing infections and maintaining health. Less bacterial disease = less use and need for ABs = less AB resistance developing.

What is worthwhile knowing about pathogen management?

First, some general pathogen management principles will be presented from the state-of-the-art (SoA) database, such as disease surveillance and disease prevention. Examples from each species will then be presented – cattle (dairy, calves, and beef), dairy sheep, pigs, and poultry.

International surveillance systems

Surveillance systems that collect and monitor disease outbreak information are a vital part of early warning systems and successful responses to disease outbreaks. Antimicrobial resistance (AMR) is a global public health threat with total mortality of 700,000 human cases per year[441]. A lack of comprehensive global AMR surveillance data and an over-reliance on an indicator-based surveillance system has limited the early detection of emerging AMR threats and trends. An open-source database called '*EpiWATCH outbreak*' has been used to retrieve

AMR outbreak reports[441]. Between August 2016- March 2020, using keywords such as ‘resistance’, ‘resistant’, ‘superbug’, ‘bugs’, ‘MRSA’, EpiWATCH identified reported AMR outbreaks quicker than an indicator-based surveillance system, as well as outbreaks by pathogen, including some not monitored by the World Health Organization. Also, it identified information on both colonised and infected cases. Thus, open-source data from EpiWATCH can complement an indicator-based surveillance system for strengthening AMR surveillance.

Financial resources may limit the number of samples that can be collected and analysed in disease surveillance programmes. When the aim of surveillance is disease detection and identification of case herds, a risk-based approach can increase the sensitivity of the surveillance system. In a paper titled *‘Application of network analysis parameters in risk-based surveillance – Examples based on **cattle** trade data and bovine infections in Sweden’,* the association between two network analysis measures (i.e. 'in-degree' and 'ingoing infection chain') and signs of infection was investigated[217]. Results showed that compared to completely random sampling, these approaches increase the number of detected positives, both for Bovine Corona Virus and Bovine Respiratory Syncytial Virus in the study population. It is concluded that network measures for the relevant time period based on updated databases of animal movements can provide a simple and straight-forward tool for risk-based sampling.

Vaccines

Development and implementation of a health management plan is the cornerstone of a profitable farm; prevention of microbial diseases by means of vaccination is an integral part of such a plan. This article reviews and discusses vaccination programs and latest advances in development of vaccines against diseases that cause major economic losses in **small ruminants**[421].

Vaccines can help minimize the need for ABs by preventing and controlling infectious diseases in animal populations and are therefore central to the future success of livestock production. Various studies have demonstrated that their use can lead to a significant reduction in AB consumption, making them promising alternatives to ABs. To be widely used in food-producing animals, vaccines must be safe, effective, easy to use, and cost-effective. Although vaccines have the potential to improve animal health, safeguard agricultural productivity, and reduce AB consumption and resulting resistance risks, targeted research and investment are needed to realize that potential. Vaccines may also have some health and welfare risks for the animals (side effects; fear and pain related to the injection). In this article[325], an expert panel discusses the opportunities, challenges and needs for the development of vaccines that may reduce the need for AB use in animals.

An article highlighting new approaches in vaccine science and potential solutions for the development of vaccines as alternatives to ABs in food producing animals[326] describes promising breakthroughs to overcome vaccine limitations, including new biotechnology techniques, new oral vaccine approaches, novel adjuvants, new delivery strategies based on bacterial spores, and live recombinant vectors. They also include new in-ovo vaccination strategies and strategies that simultaneously protect against multiple pathogens. However, translating this research into commercial vaccines that effectively reduce the need for ABs, improve animal health and protect agricultural productivity will require close collaboration among stakeholders and targeted investment in research and development.

Vaccination is an integral part of One Health strategies against infectious and often zoonotic diseases. Using EPRUMA best practice guidelines[163], EPRUMA partners wish to raise awareness on the benefits of vaccination and recommend best practices for vaccine use to ensure optimal animal health. These best-practice recommendations also aim to complement existing guidelines on vaccination, which are available in many European countries (France**,** UK - RUMA**,** Spain **-** Vetresponsable**,** Belgium - AMCRA**).**

In '*Time to Vaccinate: The importance of preventive health and vaccination programmes in ruminant production*'[244], the authors – MSD Animal Health - discuss the role of the immune system in livestock production. They define immunity as an organism's ability to resist contracting and succumbing to disease. Immunity can be innate or acquired through previous infection or vaccination. A strong immunity is therefore crucial to maintain health in our current livestock production, where multiple types of pathogens are continually challenging the health, welfare and productivity of our animals. Vaccinations play a key role in optimizing the animal's ability to resist disease. On a population basis, vaccinations have led to the eradication and control of many diseases (e.g. Rinderpest) and are indispensable tools to prevent potentially dangerous infectious diseases and to maintain animal welfare and productivity.

Biofilms

All microbial species can form or colonise biofilms (microscopic layers of bacteria coating pipes/surfaces etc.) under certain stress conditions, nutrient concentration, colony size, and degree of confinement. The role of biofilms in AMR makes it a key challenge to tackle in reducing the spread of resistance. A webinar organised by *Lallemand and Unitec*, described a method to combat the formation of pathogenic biofilm exploiting the antagonism between bacteria[364]. LalFilmPRO, can be used to reverse the formation of biofilm, using the same weapons as bacteria to extend and enhance the hygiene protocol. The novel and yet to be widely validated technology is based on the use of specific bacterial strains with antagonistic efficacy and high adherence power colonising an environment quickly and uniformly, which can inhibit and antagonise the growth of other species.

The following sections describe species-specific examples from the database regarding pathogen managment.

Cattle

Ensuring animals have access to clean water and a clean environment not dominated by infection-causing pathogens, is essential in the fight against disease and reducing the need to treat sick animals with ABs. Pruex is a UK based company that makes additives for water and bedding and is being used widely by farmers to reduce mastitis, foul of the foot, calf scour and pneumonia[12]. One Scottish **dairy** farm has applied Pruex protocols with the aim of ensuring dry bedding, clean air, clean feet and quality water. Since they have worked with Pruex products they have reduced the environmental challenge their animals face from disease causing agents. More studies using Pruex are warranted and welcome.

A paper from Italy details different strategies for reducing AB use on dairy farms including the use of immunomodulators[60]. The authors say the need for AB treatments on **dairy** farms can be reduced by a combined intervention scheme based on: (1) timely clinical inspections, (2) the assessment of animal-based welfare parameters, and (3) the use of predictive laboratory tests.

The following examples detail different approaches to managing mastitis using veterinarians, diagnostic technology, and prompt intervention.

Being able to rapidly detect mastitis and the causative pathogen is essential to reduce ABs in **dairy** farming. A farmer-led field lab, coordinated by *Innovative Farmers* in the UK aims to demonstrate that following appropriate training, farmers are capable of determining the causative agent of mild or moderate clinical mastitis using the *Vetorapid™* system and can deliver treatment based on the results[2]. Providing dairy farmers with a consistent procedure for typing bacteria rapidly on-farm has the potential to reduce AMU in lactating cows by up to 50%.

In Denmark**,** diagnosis and control of mastitis in dairy cows is led by the Consultant Veterinary Surgeons of The Danish **Dairy** Board[19]. Prevention and control of mastitis is implemented by means of a close cooperation between The Danish Dairy Board, The Mastitis Laboratory, the dairy farmer and the local veterinary surgeon. The prognosis and eventual strategy for treatment should be based upon clinical examinations and test results as well as the owner and vet's knowledge of the individual cow in a collaborative manner.

When clinical mastitis has been diagnosed in a quarter, a sterile milk sample should be taken to determine which bacteria are responsible. This will help with implementing specific mastitis control measures. **Cows** should be sampled as soon as mastitis is detected, preferably before milking is commenced. A printed resource from *Zoetis* gives farmers an easy-to-use checklist with images on how to prepare the udder and teats before taking a milk sample and how to do so as cleanly and aseptically as possible, so as to not contaminate the sample[147]. Another step-by-step farmer guide from *Zoetis* describes how to collect and transport milk samples for bacterial culture +/- sensitivity, as well as somatic cell count (SCC), the kit required marking and preparing the sterile pots, taking the milk sample, storing it correctly and sending it for analysis by a lab or the vet[149]. Taking milk samples is key to identifying the pathogen that is causing mastitis so that the correct control strategy can be selected and implemented. Environmental pathogens such as *Streptococcus uberis* require a different approach compared to *Staphilococcus aureus* or *E.coli*. Sensitivity testing can also help to assess whether the AB treatment protocols prescribed and administered for a clinical case are indeed effective.

The milk collected from **cows** with different types of inflammation, including mastitis, is characterized by an increased SCC (somatic cell count). A Romanian study aimed to evaluate the oxidative status in normal cow's milk and in subclinical mastitis milk using three parameters: total antioxidant capacity (TAC); levels of malondialdehyde (MDA); and levels of proteins (DNPH)[276]. Subclinical mastitis was diagnosed using an electrical conductivity method and by SCC in milk. Comparative analysis of TAC showed that the parameter was on average significantly lower for mastitis milk samples. The results describing the antioxidant status were correlated with those on lipid and protein oxidation. The average level of MDA in mastitis milk was higher compared to the normal milk. The levels of SCC, MDA, and DNPH were significantly higher in subclinical mastitis milk compared to milk from healthy cows.

In the Netherlands, a study to quantify the costs of clinical/subclinical mastitis and AB use found that the economic impact of reducing the percentage of clinical mastitis was found to be much larger than reducing the bulk tank SCC[48]. The optimal percentage of cows to be dried off with AB depends on the udder health situation, expressed as the bulk tank SCC and the occurrence of clinical mastitis. The bottom line was that Selective Dry Cow treatment (SDCT) was economically more beneficial than blanket dry cow treatment (BDCT using AB), for all types of herds studied. Economic profits of SDCT are greater if bulk tank SCC and clinical mastitis incidence are lower.

Scientists in Canada looked at the relationships between management practices used on **dairy** farms and herd SCC. A large number of management practices showed consistent associations with herd-level SCC[61] and should be the basis of udder health recommendations to dairy producers. Although some management practices have shown interesting associations with SCC, the lack of consistency observed should moderate reliance on their use. This review generates a more comprehensive understanding of the management practices influencing SCC and highlights specific areas that lack evidence of effectiveness.

A study from Belgium on preventing mastitis highlights the importance of reducing disease for sustainable **dairy** production[66]. High quality milk with low SCC is crucial for product quality for the processing industry (i.e. cheese making). This contributes to a reduction in food loss and food waste along the supply chain. The risk to human health from AMR and zoonotic disease spread also threatens dairy sustainability. This study uses data collected at country level on mastitis therapy and examines strategies to improve udder health for sustainable dairy production along the whole supply chain.

A comprehensive and often-cited review called *'Treatment of clinical mastitis: Using antimicrobial susceptibility profiles for treatment decisions'* evaluates the role of antimicrobial susceptibility testing in mastitis treatment[69]. There has been renewed interest in optimizing treatment protocols to better target AB administration, with substantial reliance on susceptibility testing of bacterial isolates from cows with clinical mastitis. This is despite treatments often being based on availability of labelled drugs, clinical signs in the **cow**, milk culture results for previous mastitis outbreaks in the herd, experience of treatment outcome in the herd, treatment cost, and withdrawal times for milk and slaughter. This review recommends 4 steps when selecting an AB to treat clinical bacterial mastitis: (1) appropriate spectrum of activity; (2) reaches the site of infection; (3) appropriate duration; and (4) avoids adverse effects and residues.

When mastitis incidence increases, either infection pressure has increased, or the **cow**/herd immunity has decreased. This usually indicates that farm management is not optimal. In a Dutch study by Jansen[70], the authors demonstrated that mastitis incidence can be explained by farmers' attitudes rather than self-reported behaviour. The variation in BMSCC (bulk milk SCC) value is best explained by (1) farmers' normative frame of reference about mastitis, (2) farmers' perceptions about the control of mastitis and (3) the perceived effect of a BMSCC penalty level. The variation in clinical mastitis is best explained by farmers' perceptions about mastitis control and the perceived effect of a BMSCC penalty level and the frequency of contact with others.

Trials from the UK and across the world have shown that **cows** with no evidence of existing infection within the udder can be successfully dried off with only a teat sealant[490]. The use of AB dry cow therapy can then be targeted to only those cows with evidence of infection at dry off—usually indicated by a high somatic cell count in late lactation.

In the UK, blanket AB dry **cow** therapy (BDCT) used to be commonly prescribed for many years. An alternative strategy gaining more traction is Selective Dry Cow Therapy (SDCT) whereby a teat sealant is administered instead of AB therapy to cows with a low probability of infection. Switching from BDCT to SDCT can significantly reduce AB use. Initiatives that seek to alter vets' perceptions of the risks associated with switching to using SDCT are likely to prove useful in facilitating this change[74]. The results also suggest senior vets should take a leading role in helping farms adopt SDCT. When considering how best to make a change from BDCT to SDCT, the authors propose a broad approach that clearly recognizes that the issues blocking this change are very different for vets at different stages of their career. Vets must work together to promote switching to SDCT where appropriate, and senior vets must take the lead.

Test-day SCC before drying off and after calving were used to determine **cow**'s udder health across the dry period and to study the impact on the performance in the next lactation[335]. Test-day data before drying off were explored to evaluate their diagnostic ability to detect noninfected cows at the time of drying off in 15 commercial dairy herds in Belgium with an adequate udder health management. The authors confirmed that SCC information via milk recording is capable of predicting the absence of intramammary infections (IMI) with major pathogens at dry-off, yet (an estimate of) the herd prevalence of subclinical mastitis, the cow's milk yield and parity impact the estimates of the sensitivity, specificity and predictive values to some extent. It was concluded that implementing SDCT to reduce AB use on commercial dairy herds, using strict selection criteria and test-day information, is possible without jeopardizing udder health or milk yield.

An output from the EU project '*EuroDairy*' describes how the Dutch **dairy** sector reduced AB use in dairy farming [97]. Between 2009 and 2016, usage decreased by 48%. SDCT has made an important contribution to this reduction. Preventive AB use for drying off dairy cattle is no longer allowed. The protocol uses the SCC per animal up to 6 weeks prior to drying off. If the SCC number is below 50,000 (cows) or 150,000 (heifers) per ml, no ABs may be used. If the cell number is higher, only a first-choice AB may be used in accordance with the farm health plan. The use of 2nd and 3rd choice ABs (i.e. highest priority critically important ABs) follows strict regulations, and is only allowed after additional testing. This resource also contains a farmer's experience with omitting the dry off period.

A Dutch study that evaluated the effect of 2 different communication strategies to improve udder health management showed that **dairy** farmers' management practices can be improved when both the aim of the strategy and farmers' motivational differences to work on udder health are considered[81]. When aiming at complex issues such as udder health, the traditional central route using educational tools seems to be most effective in reaching the motivated farmers. In addition to the central route, the peripheral route can be applied to influence farmers' behaviour by including implicit persuasion techniques instead of arguments in campaigns. This route is especially effective for single management practices and when aiming at a less complicated message. To reach as many farmers as possible, both communication strategies should be used. The communication strategies described in this paper[81] are examples of how management practices to control mastitis can be effectively communicated to farmers, which can be used in optimizing future programs to control and prevent diseases.

A UK field trial was conducted on a farm known to have resistant *Escherichia coli*, in order to understand if feeding **calves** with waste milk with AB residues could lead to detecting more resistant bacteria in the faeces of calves[84]. The findings of this study indicate that feeding waste milk with AB residues on this farm increases the number of resistant bacteria shed in the faeces. Resistant *E. coli* persist for longer after weaning in calves fed waste milk with AB residues. These findings are applicable to the situation observed on this farm but may differ on other farms depending on contents of the waste milk or level of contamination. Still, ABs used on this farm were commonly used on dairy farms in in England and Wales, so results shown can give an idea of what to expect in those types of farms.

Methicillin-resistant Staphylococcus aureus (MRSA) can lead to serious disease in humans, and since MRSA is often found in livestock, this could potentially have a large impact on public health. Farmers and veterinarians are especially at risk due to their close contact with animals. A Swiss study demonstrated a dramatic increase in MRSA prevalence in Swiss **pigs**, from 2% in 2009 to 44% in 2017[86]. Sequencing of the bacterial genes allowed the authors to show a close association between farmer and pig strains as well as veterinarian and **horse** strains, indicating that pigs and horses are likely to be a source of human colonization. In this study it was also shown pig 'spa t011' strains are probably less likely to colonize humans than are pig 'spa t034' strains. This research may provide a basis for a more accurate risk assessment and preventive measures.

Infectious Bovine Rhinotracheitis (IBR) is caused by *Bovine herpesvirus 1* (BoHV-1), a highly infectious virus which spreads both directly (animal to animal and over short distances through the air), and indirectly (on clothing and equipment). Infection in naïve animals can result in respiratory signs, fever, milk drop and abortion. Bulk milk antibody testing shows that many **dairy** herds in the UK are chronically infected. Animals exposed to the virus become lifelong carriers and in times of stress, e.g. at calving, can start shedding the virus. They may/may not show signs of disease at this time, but they can infect others in the herd. A single dose of Rispoval IBR Marker Live vaccine followed up to 6 months later with a single dose of Rispoval IBR Marker Inactivated, allows for an annual vaccination programme using a single dose of Rispoval IBR Marker Inactivated. A checklist from *Zoetis* details one example protocol for vaccinating herds against IBR[152].

Johne's disease is a chronic intestinal disease caused by *Mycobacterium avium subspecies paratuberculosis* (MAP). There is no treatment or cure for Johne's disease and infected animals will scour, waste away and eventually die if not culled. The progression of the disease is usually very slow with most animals becoming infected as young **calves** (often in the first

24 hours of life) but not becoming clinically ill until they are adults. It is a disease that causes considerable economic losses through decreased productivity and increased wastage of adult **cattle**, as well as the cost of monitoring, diagnosis and control. There is a strong association between Johne's disease and production problems – with Johne's cows being much more likely to have poor yields, mastitis, lameness or high SCC counts, all of which lead to premature culling. A tool developed in the UK by National Milk Recording provides a guide to understand and control the disease on dairy farms[155].

Due to the nature of Johne's disease, accurate identification of infected animals is often difficult, especially in the early stages of the disease. Infected animals in the early stages are unlikely to shed the MAP organism or produce a detectable antibody response. In the later stages of Johne's disease, cows will often intermittently shed MAP and exhibit peaks and troughs of antibody production until reaching the clinical stage of the disease. A suite of tools developed by the UK's National Milk Recording can help vets and farmers understand Johne's testing schemes[154] and focuses on two major principles: identifying the MAP organism itself and looking for the animal's antibody response to the disease.

For James Smith, winner of the UK's National Milk Record's 2018 Herdwise award, reducing Johne's disease burden across his 240-cow organic Holstein Friesian herd in Chippenham, UK saw Johne's positive **cows** reduce from 35% of the herd to 15% in two years[141]. Since June 2016, this significant reduction has included the introduction of several herd management practices, such as installation of a pasteuriser and Johne's testing done quarterly via milk samples taken as part of the 'Herdwise Johne's screening scheme'. If a cow has a positive result above 60%, it is culled at the end of that lactation. Cows with very high readings, often termed 'super shedders', are culled as soon as possible. *"The long-term goal is to eliminate Johne's completely, but realistically this will take several years so it is vital we have the management strategies in place to achieve this."*

Bovine herpesvirus 1 (BoHV-1) and bovine viral diarrhoea (BVD) virus cause diseases of **cattle** with a worldwide distribution. A study from Northern Ireland described herd-level BoHV-1 and BVDV seroprevalence (based on testing of pooled sera) and control measures on farms including vaccine usage[243]. The results from this study indicate that the true herd-level seroprevalences to bovine herpesvirus 1 and bovine virus diarrhoea virus in non-vaccinating herds in Northern Ireland in 2010 were 77.3% and 98.4%, respectively. The study will assist in guiding regional policy development and establish a baseline against which the progress of current and future control and eradication programmes can be measured.

Three vaccination protocols against Bovine Respiratory Disease (BRD; Bovilis Bovigrip®) for young **Charolais cattle** were conducted in commercial feedlots in France to identify respective benefits[357]. The average daily gain was significantly higher when animals were completely vaccinated (2 shots) at breeding farms (early vaccination) compared to those where part of the vaccination was done at the assembly centre (intermediate vaccination). The number of cattle which were observed as sick by farmers was low on average, as well as the proportion which were treated by them (<15%/lot) but no significant difference could be demonstrated between protocols. In the context of reducing medication inputs, this experiment suggests the benefits of vaccinating cattle was most noticeable at the breeding farm to prevent and decrease BRD incidence in feedlots.

A Belgian study aimed to develop and validate 2 protocols (for use on-farm and at a central location) for the reduction of *Mycobacterium avium ssp. Paratuberculosis* (MAP) in colostrum while preserving beneficial immunoglobulins (IgG)[334]. The on-farm protocol was based on curdling of the colostrum, where the IgG remain in the whey and the MAP bacteria are trapped in the curd. The semi-industrial protocol was based on centrifugation, which causes MAP to precipitate, while the IgG remain in the supernatant. The effect of the colostrum treatment on the nutritional value and palatability of the colostrum and the IgG transfer was assessed in **calves**. The treated colostrum had no negative impact on animal health, IgG uptake in the blood serum, milk, or forage uptake. Two protocols to reduce MAP in colostrum (for use on-farm or at a central location) were developed and both methods preserve the IgG which is vital for the young calf's passive immunity.

Respiratory diseases are frequent in **calves** due to weaning stress, transport and environmental changes. The aim of a Romanian study was to isolate bacteria from 30 calves with respiratory disorders and test their antimicrobial susceptibility[279]. The study carried out on nasal discharge samples collected from calves with respiratory disorders and their antimicrobial profile testing led to the following conclusions: 1) Low susceptibility to Florfenicol is caused by previous treatments when this molecule was excessively used and without prior sensitivity testing. 2) Cefquinome may represent an emergency therapeutic AB for respiratory infections in calves, but the administration should always be preceded by susceptibility testing of the isolates.

The impact of concomitant vaccination of **cows** to protect the young **calf** against neonatal diarrhoea and respiratory diseases was tested in a French study[492]. Within the first 45 days, calves from vaccinated cows received 1 AB treatment less than other calves. The impact was visible from the arrival at the fattening unit: 1.8 less risk for vaccinated cow calves to show clinical respiratory disease, and a higher weight (1 kg) at equivalent ages. As a consequence, the mortality of calves from vaccinated cows was lower.

For suckling **dairy calves**, different management routines to ensure sufficient colostrum intake are used: visual assessment, hand feeding supplemental colostrum or assistance. However, not much is known about the efficacy of these methods to prevent failure of passive transfer (FPT). In a Norwegian study - '*A cross-sectional study of suckling calves' passive immunity and associations with management routines to ensure colostrum intake on organic dairy farms'*, the prevalence of FPT among all suckling calves was high, and comparable to that reported from Norwegian calves in conventional herds that are separated from the dam and fed colostrum artificially[71]. Securing high colostrum quality is an important preventive measure of FPT in suckling dairy calves. The results indicate that for calves capable of finding the udder and suckling independently, there is no direct benefit of routinely hand-feeding colostrum, although herd level factors may play an important role. Herds practicing suckling (indeed all farms rearing calves) need to systematically address all three important factors to ensure passive transfer of immunity: time from birth, colostrum quantity and colostrum quality.

A trial in France to test dam vaccination on calf protection involved a survey of 36 **dairy** farmers on their perception of this practice: 6 months before the trial (2017), 77% of them had a preventive perception of the vaccination but they associated it to rather curative practices[493]. Their participation in the trial has improved their understanding of calf passive immunity and of the role of colostrum into this process (75% of answers), but also the importance of the delay for colostrum intake (72%) and of the quality of colostrum (65%). After the trial, the farmers declared to be determined to change their practices regarding the provision of colostrum to improve the health of calves at farm. These results confirmed the benefits of providing practical information to facilitate the changes in attitude and practices.

A case/control study was set up in nine **cow-calf** operations and four fattening units to compare preconditioned and control cattle in a French context[497]. The preconditioning protocol consisted of weaning calves 50 days before sale, with adaptation to solid feedstuff and housing. A trivalent vaccine protocol [BRSV, BPI3, *Mannheimia haemolytica* (formerly *Pasteurella haemolytica*)] and vitamins and micronutrient supplementation were also implemented in order to improve the immune response to respiratory diseases. Contrary to what was expected, disease incidence and lung lesion score were higher for preconditioned **young bulls** compared to controls. These results could be explained by the epidemiological context of fattening units, poor housing conditions in cow-calf herds and individual immune competence, in relation to immune status and previous vaccination. Pathogens detected in fattening units (e.g. BCoV and *Pasteurella multocida*) were essentially different from the vaccine valences. This study identifies critical parameters for the settlement of preconditioning programs and highlights the necessary adaptation to local conditions and husbandry factors.

Sheep

Lameness constitutes a major animal welfare and economic challenge across the **sheep** sector, costing an estimated £24 million to the UK industry[290]. The high volume of AB currently used to tackle the estimated 9 million lameness cases that occur annually in the UK is also concerning. The aim of this study was to develop and validate an achievable farm-level solution, the Five Point Plan, to reduce lameness levels to Farm Animal Welfare Council (FAWC) targets. The Five Point Plan has five action points that support the animal in three different ways: building resilience, reducing disease challenge and establishing immunity. It was then implemented on a UK sheep farm over a four-year study period (2009–2013). Lameness prevalence across the study flock was measured monthly by a single observer using a simple 6-point locomotion score. The results show that lameness reduction is achievable within a relatively short time scale but does require long-term commitment in order to sustain success.

A total of 160 **ewes** on one farm in England were studied for 18 months[291]. Cases of footrot and interdigital dermatitis in individually identified sheep and treatment and flock control measures were recorded. In this one flock, cases of footrot and interdigital dermatitis were linked and associated with trimming of feet. They were also negatively associated with the use of ABs and topical AB sprays in the first +/- second 2-week period. These results suggest 1) that footrot and interdigital dermatitis are infectious diseases that can be controlled, in part, through the use of ABs, which acts to reduce the infectious period of diseased sheep and 2) that routine trimming of diseased and healthy feet increase disease, through environmental contamination +/- through increased susceptibility of sheep with recently trimmed feet.

Further research from the UK looked at how veterinary surgeons' beliefs regarding effectiveness of two treatments for footrot changed following a review of the evidence[292]. There was considerable variety in veterinary surgeons' beliefs before they listened to the review. After hearing the evidence, seven participants quantifiably changed their beliefs. The results suggest that a substantial amount of the variation in beliefs related to differences in veterinary surgeons' knowledge. Two key findings from the qualitative data were: (i) veterinary surgeons believed that farmers are unlikely to actively seek advice on lameness, suggesting a proactive veterinary approach is required (ii) more attention could be given to improving the way in which veterinary advice is delivered to farmers. In summary this study has revealed that the evidence that currently exists can change veterinary opinion and improved transfer of research knowledge into veterinary practice is needed.

Another UK based study was conducted in 2008 where 809 English **sheep** farmers were asked to identify six common foot lesions; only 20% of farmers named all six lesions correctly[299]. This study highlights the necessity of vets in advising and educating producers about sheep lameness. Foot lameness in sheep can be attributed to infectious or non-infectious causes. The three infectious causes of lameness covered in this article are:

- Ovine interdigital dermatitis (OID), often referred to as scald;
- Footrot (infectious pododermatitis);
- Contagious ovine digital dermatitis (CODD).

Non-infectious causes of lameness are less common and include:

- White line degeneration;
- Foot abscess;
- Toe granuloma.

Getting an accurate diagnosis is essential in advising on prevention, control and treatment. Often, one or more conditions are present on a farm. Lesions can be in the early or chronic phase, where they can appear grossly very different, so sufficient animals need to be examined to achieve a diagnosis[299].

Another lameness study aimed to evaluate the effectiveness of ozone therapy and platelet rich plasma (PRP) in the treatment of acute footrot[300]. Ten **sheep** suffering from footrot were treated and compared to a control group of 5 healthy sheep. Treatment consisted of local ozone application and then with non-healing cases, the application of PRP. Complete recovery was achieved after local ozone treatment in 6/10 sheep. The remaining four animals also healed after the subsequent PRP therapy. Results showed that ozone treatment did not cause major blood or inflammatory changes and the local application of ozone and PRP proved to be an effective footrot treatment that avoids the use of ABs/ disinfectants. However, due to the relatively high costs and time requirements, it is potentially most suitable for smaller farms.

In the UK, 160 **sheep** farmers were asked via questionnaire about their current management of footrot and their satisfaction with it[301]. Farmers satisfied with current management reported <= 5% lameness. Farmers reported treating lame sheep within 3 days of first seeing them lame, and those with footrot/scald with parenteral ABs. Farmers dissatisfied with their management reported >5% lameness. These farmers practised routine foot trimming, foot-bathing and vaccination against footrot. Whilst 89% of farmers said they were satisfied with their management practice regarding footrot, >34% were interested in changing what they did. Farmers reported that ideally, they would control footrot by culling/isolating lame sheep, sourcing replacements from non-lames, trimming feet less, using less ABs and using vaccination more. Foot-bathing was common, linked with dissatisfaction but also listed highly as ideal management practice. Some of the ideal management interventions aligned with best practice but others contrasted with it suggesting cognitive dissonance was present.

In another study, the goal was to understand how key players in the UK **sheep** industry recommended treating footrot and tested whether reviewing the evidence surrounding treatment of footrot changed their beliefs[302]. All participants recommended use of AB injection but only four recommended not foot trimming feet with footrot. During discussions, participants stated that parenteral ABs had always been recommended as a treatment for footrot but that the new research clarified when to use them. In contrast, it was highly novel for them to hear that foot trimming was detrimental to recovery. After hearing the evidence, seven of the eight key players would recommend avoiding foot trimming. Some were resistant to changing beliefs despite hearing the evidence. Three months after the workshop, three participants stated that they now placed greater emphasis on rapid individual AB treatment of lame sheep and not foot trimming feet.

In **small ruminants**, management tools for the control of mammary infections must be reviewed, considering animal specificities as well as management, equipment and work organization. A French study integrated observation of the work of breeders and advisors, various innovative technological solutions or automated recordings now available or in development as diagnostic tools (molecular bacteriology, cell counts, infrared spectra, clinical examination of the udder and the teat), and milking ability and milking conditions[426]. In a context of AB-use reduction, criteria for the selection of animals to be cured (or culled) have been proposed. New phenotypes have been explored for a better understanding of the risk factors associated with milking. Finally, after studying the genetic progress and economic impact, the inclusion of new traits in selection schemes was carried out (i.e. somatic cell counts) or proposed to improve the resistance of animals to mammary infections.

Pigs

AMU in **pig** farming is influenced by a range of risk factors, including herd characteristics, biosecurity level, farm performance, occurrence of clinical signs and vaccination scheme, as well as farmers' attitudes and habits towards AB use. So far, the effect of these risk factors has been explored separately. A multi-institute study aimed to investigate the relative importance of all these risk factors in a sample of 207 farrow- to-finish farms from Belgium, France, Germany and Sweden[132]. The occurrence of clinical signs, especially of respiratory and nervous diseases in fatteners, was one of the largest contributing risk factors in all four countries, whereas the effect of the other risk factors differed between countries. In terms of risk management, it suggests that a holistic and country-specific mitigation strategy is likely to be more effective.

Chlortetracycline and the macrolide tylosin were identified as commonly used AB for growth promotion and prophylaxis in **pig** production. Resistance to these ABs was measured throughout the waste treatment processes at five pig farms in the US by culture-based and molecular methods[447]. Conventional farm samples had the highest levels of resistance with both culture-based and molecular methods and had similar levels of resistance despite differences in AMU. The levels of resistance in organic farm samples, where no antimicrobials were used, were very low as assessed by a culture-based method targeting faecal streptococci. The levels of tetracycline and MLSB (Macrolide-lincosamide-streptogramin B) resistance remained high throughout the waste treatment systems, suggesting that the potential impact of land application of treated wastes and waste treatment by-products on environmental levels of resistance should be investigated further.

Diarrhoea in **piglets** can be caused by several pathogenic agents, including *Campylobacter* spp., *Clostridium perfringens, Escherichia coli*, Salmonella spp., Rotavirus group A (RV-A), coronaviruses (transmissible gastroenteritis virus—TGEV; porcine epidemic diarrhoea virus—PEDV), as well as by nematode and protozoan parasites. However, most studies have focused on a few or only one agent and consequently our understanding of the relative importance of pathogens, their interactivity and other factors may have strong biases[472].

The effect of vaccination against neonatal diarrhoea is not always high because immunity is also based on the intake of colostrum[91]. An average **sow** produces enough colostrum for 12 piglets but the average litter size is now 15 piglets. One way to improve colostrum supply is split suckling. Industry actors in the Netherlands describe that in the case of neonatal diarrhoea in piglets, it is almost always necessary to take steps on several fronts, especially pertaining to hygiene. If the piglets do develop diarrhoea, it may be effective to provide the sows with acid and cola, then offering water and food, with the third step the possible application of vaccinations and/or ABs.

A particularly economically important disease in pigs is Post-weaning diarrhoea (PWD) caused by *Escherichia coli.* It is affecting pigs during the first 2 weeks after weaning and characterized by sudden death or diarrhoea, dehydration, and growth retardation in surviving piglets[473]. Furthermore, many stress factors associated with the weaning period, such as removal from the sow, dietary changes, adapting to a new environment, mixing of pigs from different litters and histological changes in the small intestine, may negatively affect the response of immune system and lead to an intestinal gut dysfunction in **piglets**.

A Belgium study investigated the effect of vaccination against *Mycoplasma hyopneumoniae* on its transmission in nursery **pigs** under field conditions[296]. Seventy-two pigs were randomly placed at weaning into vaccinated (V) and non-vaccinated (NV) groups. Animals in the V group were vaccinated at 3 weeks of age with a commercial *M. hyopneumoniae* bacteria vaccine. Broncho-alveolar lavage fluid taken at weaning and at the end of the nursery period was assessed for the presence of *M. hyopneumoniae*, and the reproduction ratio of infection (Rn) was calculated. The study indicates that vaccination does not significantly reduce the transmission of this respiratory pathogen.

Vaccination is also an effective means to prevent and reduce problems with Porcine Intestinal Adenomatosis (PIA)[168]. PIA leads to loss of growth and vaccination can reduce this. *MSD*

Animal Health has manufactured a PIA vaccine. The vaccine can be administered from 3 weeks of age and provides protection from 4 weeks up to 21 weeks after vaccination. Vaccination results in fewer deaths, lower infection pressure and less diarrhoea caused by Lawsonia infection. The vaccination has resulted in positive experiences in practice.

Subclinical infections with *Salmonella Typhimurium* occurs frequently in pigs, constituting a risk for human salmonellosis. In this Belgium study, an attenuated *Salmonella Typhimurium* vaccine (Salmoporc®, IDT Biologika) was evaluated in three **pig** herds[161]. The excretion of *Salmonella Typhimurium* field strain was low and similar between farms and production cycles. Vaccination of either sows and piglets, sows and fattening pigs, or in piglets only, resulted in a smaller number of lymph nodes positive at slaughter, in pigs in the second production cycle only. Marked reduction of positive lymph nodes at slaughter happened after vaccination of sows and piglets. The vaccine strain was detected in the lymph nodes of 13 pigs at slaughter. Because of study limitations, results should be interpreted with care. Nevertheless, in sows and piglets (preferred), sows and fattening pigs, and piglets only, vaccination can (to some extent) support the control of *Salmonella Typhimurium* infections.

In a study from Denmark, liquid pig manure (n=305) and sewage sludge (n=111) - used as agricultural fertilizers between 2002 and 2005 - were investigated for the presence of *Escherichia coli*, *Enterococcus faecalis* and *Enterococcus faecium*[445]. Bacteria were tested for their resistance against 40 chemotherapeutics, including several "reserve drugs". E. coli (n=613) from pig manure were at a significantly higher degree resistant to streptomycin, doxycycline, spectinomycin, cotrimoxazole, and chloramphenicol than *E. coli* (n=116) from sewage sludge. Enterococci from **pig** manure were significantly more often resistant to high levels of doxycycline, rifampicin, erythromycin, and streptomycin than *Ent. faecalis* (n=44) and *Ent. faecium* (n=125) from sewage sludge. Significant differences in enterococcal resistance were also seen for tylosin, chloramphenicol, gentamicin and more. High rates of (multi-) resistant bacteria in pig manure emphasize the need for a prudent - cautious - use of ABs in farm animals.

Poultry

Colibacillosis prevention in chicken farms should be maximized as this is a disease commonly treated with ABs. Understanding the psychological and social context in which farmers operate can have an impact management of the birds. In France, an initial survey (qualitative study) was carried out involving 14 conventional **chicken** production farmers[264]. Afterwards, 75 western France farmers replied to a quantitative questionnaire based on results from the previous survey. The survey revealed that 85% of farmers had insufficient knowledge about colibacillosis. The major incentives to prevent avian colibacillosis were the desire to improve farm income and to reduce AB use. Statistical analysis showed five farmers profiles. Advice should be adapted according to these profiles:

1. experimented little motivated farmers
2. young and independent farmers
3. farmers with other priorities
4. motivated with small chicken house farmers
5. risk taking farmers

In another study, a 28-day experiment from the US showed promising results in the recovery of **broiler chicks** after a challenge with mild coccidiosis infection (*E. tenella*)[350]. The chicks were fed a diet containing an additive that boosted their immune response to the parasite and an additional antioxidant to mitigate tissue damage to the gut from an excessive immune response. Both the chicks on the control diet (without the new additives) and the chicks receiving the treatment were challenged with a mild coccidiosis infection and did not differ in overall performance. However, the chicks on the treatment diet showed faster recovery and better daily gain instantly after the infection with the parasite. This led the researchers to believe that chicks recently infected would benefit more from this innovative dietary supportive treatment.

A French company, Altitude, has developed the *H@tch Vet* Expert application[31]. This app, developed for the Merial and Chêne Vert Conseil laboratories, is intended to be used during audits in hatcheries. Veterinarians fill out a predefined questionnaire in the application during their visit to the farm. This questionnaire, the responses of which appear in the form of notes, text, multiple choice questions, etc. then makes it possible to create statistics but also to benchmark the farms in relation to previous audits or in relation to other farms of the same type. The questionnaires and statistics can be managed from the app but also from the website.

5.3. Housing and welfare

Highlights

- Modern farming is associated with substantial welfare problems that also have an impact on disease status and the development of antimicrobial resistance (AMR).
- Animal welfare is the quality of life as perceived by the animals themselves.
- Health is an important welfare need.
- Stressed animals have a reduced immune function, increased disease susceptibility and are therefore likely to need more AMU to treat diseases and hence develop AMR.
- Automated sensors (e.g. of climatic conditions) and improved early-life management can considerably contribute to improved production, health and welfare of livestock.
- Happy animals may be more resilient, and they can make farmers happy too.

What is housing and welfare?

Aspects of housing and welfare concerns could be seen respectively as the hard and soft aspects of animal production. That is to say, housing refers to the hardware of the system in which the farmers aim to achieve the animals' capacity to produce as efficiently as possible. Housing constitutes an important part of an animals' physical environment, which determines to a large extent the animals' health and welfare status. Animal welfare may be defined in different ways. For members of the general public animal welfare is often related to a more 'natural' living, though nature is not always good for welfare. Some welfare scientists have proposed to define welfare in terms of (the measurable aspects of) biological functioning, but this definition also poses issues since biological functioning (survival and reproduction) per se is, for example, also a property of plants, which normally are not considered to have a welfare state; and on the other hand, poor welfare can be present in animals that are highly (re-)productive. Most animal philosophers and many welfare scientists, define animal welfare in terms of feelings or affective states, e.g. as the quality of life as perceived by the animals themselves (Bracke et al., 1999). Feelings have evolved to help animals deal with a variable environment, and thus support animal-specific biological functioning. Welfare problems of modern farming practices often relate to the fact that animals are kept in environments that differ very much from the environment in which they have evolved and to which they are adapted. Keeping large numbers of animals in crowded and barren conditions, as well as other management decisions associated with intensive farming (e.g. very high production levels (milk yield, piglets per sow per year, growth rates), early weaning of piglets or motherless rearing of calves and chicks, food restriction in gestating sows and broiler breeders), and mutilations (e.g. castration, dehorning, disbudding, tail docking and beak trimming), are often associated with stress and reduced welfare, as well as a certain risk for disease. Welfare problems, however, are not only related to intensive housing, but also to management decisions aiming for maximised production efficiency and to some forms of (very) extensive production. Welfare, then, is how the animals perceive their environment and provisions of resources (i.e. the things they need) in terms of positive and negative affect (positive and negative welfare). In that sense, welfare is inherently subjective (i.e. 'soft'), even though it is increasingly possible to measure and model animal welfare based on scientific information. Main scientific paradigms to measure welfare include, besides the study of behaviour (preferences, demand, abnormal behaviours), also the health status of the animal (e.g. skin lesions, etc.), stress levels (which can have an effect on e.g. immune function) and other aspects of biological functioning, including the levels of production and reproduction (Bracke et al., 2002). Health is also an important welfare need, as it may override (to some extent) other welfare needs. All welfare needs, including the need for health, are based on behavioural systems, like searching for and ingesting food, having social interactions, reproduction, thermoregulation, rest, exploration, body care, etc. The health need relates to so-called sickness behaviour, and it is not the health status per se that determines welfare, but how sickness is perceived by the animals and expressed as a behaviour. For example, a tumor can be aggressive (spread in the body), implying a severely reduced health status, without (at that point) affecting the welfare of the individual (yet). Conversely, a mutilation like tail docking, even when conducted adequately without any analgesia, is quite painful even though it does not have a big effect on the animal's health status (although the wound is a potential point of entry for pathogens).

How does attention to housing and welfare help reduce antimicrobial resistance?

Providing high-quality housing and good welfare conditions for animals are likely to reduce stress, improve production and reduce the need for AMU, e.g. improved climate control and improved immunocompetence will result in less disease.

Why is housing and welfare important?

Good quality housing is important because it provides the environment for both the farmer and the animals to function well. Good quality flooring is important for locomotion and resting, including the prevention of health-related disorders like lameness and pressure sores. Good insulation and shelter is important for thermoregulation and disease prevention. Adequate space is important to allow for the performance of most (natural) behaviours. Proper biological functioning, including production performance, is generally indicative of improved welfare.

By definition, animal welfare is all that matters to the animal (as it is the quality of life as perceived by the animal itself). High production and health are normally a necessary, but not sufficient, condition for good welfare. Sick animals often have reduced welfare, but good welfare also requires giving animals the opportunity to express normal and species-specific behaviours, such as rooting and wallowing in pigs, scratching, dustbathing and roosting in poultry, and grazing and ruminating in dairy cattle. While reduced AMU is an important aspect of agricultural sustainability as it serves to protect human health and thereby human-welfare interests, animal welfare is also important, in and of itself. This importance, thus, does not only the fact that good animal welfare may help to improve animal health and reduce AMU, but also because from an ethical standpoint, animals should be handled humanely and deserve a good life, a life worth living too.

What is interesting and worthwhile knowing about housing and welfare in relation to AMU and AMR?

General

As discussed earlier, the health and welfare of animals is related to housing conditions.

Main recommendations and standards have been summarized, providing a good overview of **sheep** basic needs in terms of housing design and layout[367]. For **pigs**, a study on 130 farms in 5 EU countries identified several factors that related optimal housing and farm and health management to improved sow and piglet performance[127].

Climate & climate-related emissions

In **broilers,** there are several important aspects of production that require careful consideration. Thermal models used in **broiler** production are outdated and not sensitive enough to fluctuations over time. An hourly model of heat, carbon dioxide (CO_2) and water vapor production was developed for broilers incorporating performance parameters. The model can be used for climate control and thermal design of broiler houses[259]. EU directive 2007/42/EC sets a maximum limit of 3000 ppm CO_2 for broilers (at animal height over the entire duration of the batch). Since then, CO_2 concentration sensors have been developed for use in French poultry buildings. Regarding current CO_2 sensors, measurement should be done at animal level at the end of the rearing period at a height of 80 cm +/- 20 cm, even though this may result in an underestimation of CO_2 levels in case of high CO_2 production by animals and litter. It's better to use more than one CO_2 sensor for continuous measurement in poultry barns, but the first level investment should be in a high-performance sensor and its maintenance rather than in purchasing an additional sensor[260]. As of 2010, a maximum limit of 3000 ppm CO_2 is allowed at animal height over the entire duration of the rearing period. Two out of five CO_2 sensors tested were not suitable for continuous use in **poultry** buildings. A height of 80 +/- 20 cm above floor level (between nipples and feeders) is the recommended position at the start, but this gives an underestimation at the end of the rearing phase. CO_2 heterogeneity is more marked at the start than at the end. If an additional sensor is not economically feasible, a correction could be applied. Again, using a high-performance sensor and its proper maintenance is preferred over buying an additional sensor[261].

Also **pig** farms should monitor closely their emissions. Recent gas-emission management guidelines have been specified for **pig** farms (2020)[433] (in Spanish) (see also Klimaatplatform Varkenshouderij, 2021, in Dutch). Reducing gas-emissions is possible through technical improvements to the housing system. For example, the Kempfarm system has a manure belt underneath the slats to separate urine from faeces in **pig** barns to reduce ammonia production and prevent lung problems by removing solid manure from the shed twice a day[88].

Management

The management qualities of farmers can have a significant impact on the animals' health status and the required AMU to maintain production. Better understanding of farmer behaviour is important, including farmer-vet relationships, audit and inspection dynamic, cultural ideas about farming and the role of 'good farming practices' in farmers' decision making and actions[94]. To support improved management, tools have been developed to assist the farmer. Predict and prevent by Prognostixs software, for example, supports tracking and analysing performance based on sensor data related to health and environment to inform management decisions[13] (see also the chapter on precision livestock farming). Water management guidelines have been specified in Spanish[432].

A simulator to calculate water medication and water consumption is also available[436] (in Spanish) (see also the next chapter on water management in this state-of-the-art report).

As to management decisions made in the dairy sector, bacterial load was found to vary within and between bedding materials used for **cattle** in the UK, i.e. it was higher when recycled material solids (RMS) were used compared to sand and sawdust. Teat dipping with a disinfectant and drying, prior to milking, as well as disinfecting clusters between milking different cows resulted in lower bacterial counts in milk[37].

Guidelines on **milking** control and hygiene are also available in French[491].

Another guide aims to help farmers use new **milking** technologies and automatic milking systems. It describes the various technologies available for mastitis monitoring and provides some general tips on maintaining good udder health[394]. Finally, the database contains an individual **pig**-care poster showing different signs of acute, sub-acute and chronic conditions to be recognised by the farmer[435]. Note: a number of practice guides and information sheets related to (external) biosecurity and prudent use of antimicrobials included in the database are referred to in the chapters on biosecurity and prudent use respectively.

Young stock management

Various publications draw attention to the specific management of young animals. These have been grouped by species. The Dutch **calf**-reception project, for example, aims to improve the quality of young calves on the **veal** farm, by focusing on veal farm management in the first four weeks, including the release of calves in groups[90]. On average, 14.5% of live-born **dairy heifers** fail to reach their first lactation, esp. due to pneumonia in calves aged 1 to 6 months[158]. As to respiratory health, also UK **beef calves** with healthy lungs gained 72 g/day more than those with moderate lung damage, and 202 g/day more than those with severe damage. The first two months of life are of particular importance for lifetime performance as indicated by age at first calving, first and second, lactation milk yield and longevity in the dairy herd. Careful, proactive management of the young calf is critical to maximise future productivity[158]. Dairy Australia produced a manual related to healthy **calves** and biosecurity[466,474].

Another document (in French) reviews **lamb** health and housing (from birth to fattening). It also describes good practices related to housing, including biosecurity, water, litter, farm layout, cleaning and disinfection, lighting and ventilation[363].

Rearing may also have a long-term impact on behaviour, health, and welfare of **layers**. Enriching the rearing environment with physical, sensory, and stimulatory additions can help maximize the birds' developmental potential. The impact of enrichment provided during rearing on behavioural and physiological development is reviewed. Improved behavioural opportunities (for e.g. dustbathing, perching and foraging) will improve bird welfare and probably immunocompetence, though the mechanism is poorly understood. There is a need to identify and validate practical cost-effective enrichments for on-farm use[365]. **Broilers** that hatch in the barn show reduced mortality and foot sole lesions. There were no differences in behaviour shown in the pens, but chicks responded differently to a challenge, both at a young age and at an older age[353 (in Dutch)]. An ITAVI document (in French) and protocol aims to support a good and AB-reduced start in **broilers**[30].

A directive was produced for Dutch **poultry** veterinarians concerning the start-up and management of broiler chicks in the first week of life. It concerns the prescription of ABs and practical tips and norms regarding climate, lighting, water, feed and supportive measures such as the use of pre- and probiotics, vitamins, minerals, organic acids and fytobiotics. This guide can also be used by broilers farmers to improve their start-up management[257]. **Chicks'** vocalisations in the first 3 days of life (D0 to D3; e.g. snuggle, fear, pleasure trills, but esp. comfort and distress calls) can be recorded and analysed automatically to inform the farmer about the health and welfare status of the animals. Optimal sound recording conditions include a group of ten chicks, omni-directional microphones and 2-min. recording sequences. Between D0 and D3, chicks emit short sounds with a limited frequency range (2000 - 5000 Hz)[266]. Early rearing was examined in 30 **Label Rouge poultry** flocks. Origin of the chicks had a significant impact on weight gain, flock homogeneity and mortality at 15 days of age. Specific start-up feeding equipment also appeared to influence weight gain, homogeneity and rate of pododermatitis. The latter was also influenced by litter quality[268]. A good start is important for the further development of poultry, incl. **Label Rouge broilers**, and influences the success of the batch in breeding. Following the start-up monitoring in 45 Label Rouge broiler farms (in France); two major factors were: Early feeding and watering, and good quality bedding[518].

Another important aspect of management is the handling of day-old **chicks**. The quality/robustness of these animals plays a crucial role in welfare levels, mortality and AB use. It is negatively affected by breeder age and time at the hatchery. Chicks placed at lower density, in a controlled environment in the hatchery, and with empty space between each box to improve air circulation had significantly less weight loss until arrival at the farm, increased body weight at D12 when hatched from brooding eggs collected at the end of lay, a higher cloacal temperature at D1, and a higher breast yield at slaughter, but similar mortality levels[271]. In 50 **broilers** flocks in Brittany the average mortality rate was 1.9% and 47 % of the flocks received AB treatment in the first 10 days of life. Flocks with elevated mortality rates (2.3 %) were treated with AB more often (58 % of the flocks), had more *E. coli* detected in chicks at Day 1, more lameness at Day 3 of life, generally higher CO_2 levels at Day 1 (> 3000 ppm), were located further from the hatchery (> 200 km) and did not use detergent to clean the poultry house[272].

Welfare

Pain

Post-operative pain relief (in addition to the use of CO_2 anaesthesia during the procedure) for castration of **pigs** is not legally required in the Netherlands, as it is e.g. in Germany. Pain relief should be administered half an hour before castration. Some pig farmers administer pain relief at the time of castration, but give the wrong medication or not the proper concentration for piglets. Proper pain management, however, makes the piglets recover faster. The drug costs roughly 2 cents per piglet[247]. Since mid-June 2020, Dutch **pig** farmers are conducting welfare checks for sucking piglets, weaned piglets, fattening pigs and rearing sows (collected at www.welzijnscheckvarkens.nl), particularly in relation to tail, ear and flank biting. Both animal-based and resource-based indicators are used to assess risk, improve welfare and productivity. The welfare check for pigs has been developed because it is legally required and helps demonstrate good welfare to purchasers of Dutch pigs and pork[250].

Tackling lameness in **sheep** includes correct diagnosis and prudent use (providing AB only when necessary and effective). A 5-point plan includes culling animals that are lame repeatedly/persistently, quarantining all incomers and treating affected sheep appropriately[422].

Three booklets are available in French on intervention methods on lameness in **dairy** herds based on pooling of expertise (based on approach, risk factor inventory and intervention) [475].

Guidelines have also been produced for on-farm killing of **pigs**[434 (in Spanish)].

Enrichment/improved welfare

Enriched housing was shown to reduce disease susceptibility in **pigs** (faster viral clearance in the blood, and less lung lesions and tissue damage following an experimental infection with PRRSV and *A. pleuropneumoniae*)[5]. The German welfare scheme "Initiative Tierwohl' improves relative farm performance and respiratory health of **pigs**, and is compatible with a high economic and high health performance (Uehleke et al., 2021).

Production data from six top **poultry** farms in Romania showed increased production costs (of between 1.8 and 3.4%) due to the introduction of broiler welfare rules, esp. related to lighting, fuel, mortality and labour, while savings were found for biological materials, feed and ventilation[282].

The Austrian Animal Needs Index 35L/2000 was applied to assess **dairy** welfare in a loose-housed Romanian farm using 26 indicators (related to locomotion, social interactions, flooring, light, air and stockmanship). The highest scores concerned locomotion and social interactions. The lowest scores concerned flooring and light and air. Lighting was critical (uneven lighting and low intensity values: 28-30 Lx), as were dirty outdoor areas. Addressing these issues may improve dairy welfare levels and increase milk production[297]. Following previous research showing a link between improved animal welfare, biosecurity and AMU reduction in pigs and dairy cattle, a study was conducted on 27 specialised **beef** farms over 3.5 years, reporting a need for implementing biosecurity measures and emergency management, due to the low on-farm assessment scores (24 and 39% respectively) and found also a statistically lower AMU in relation to improved welfare[449].

5.4. Water management

Highlights

- Livestock need easy access to a sufficient amount of good quality water.
- Having a source of good quality water does not necessarily mean the animals are provided with good quality water, as water quality may deteriorate between the point of entry on the farm and ingestion by the animals.
- Biofilms in water pipes may be contaminating the drinking water of livestock.
- Farmers need to pay attention to water provision (quality and quantity) for their animals.

What is water management?

Water management involves making sure animals have access to a sufficient amount of water that is of good quality so as to avoid health and welfare problems, and therefore ensure optimal production.

How does water management help reduce antimicrobial resistance?

Poor water quality increases pathogen load and reduces the ability of the individual animal and the herd/flock to maintain a good health status. Health problems lead to increased antimicrobial use (AMU), which leads to enhanced risk of developing antimicrobial resistance (AMR).

Why is water management important?

Water management is important to maintain productivity, health and welfare of the animals.

Broiler farmers' confidence in water quality is often not justified. Dutch research showed that almost 8% of nipple water was unsuitable and almost 20% was less suitable for **poultry**. Water quality deserves continuous attention, particularly when mains water is used (vs bore water hole)[101]. Acidification of drinking water of **nursery piglets** on one study farm helped reduce *E. coli* and post-weaning diarrhoea. Further work is needed to confirm the findings on production and clinical parameters[143]. Note: it is now common practice in pig production to acidify drinking water to reduce AMU.

What is interesting and worthwhile knowing about water management?

Biofilms can reduce the water quality in pipes. A scoring method was developed using pictures and objective definitions to assess the effect of purging water pipes to reduce biofilms. The first results highlight the importance of cleaning the pipes before starting flocks. Frequent purging seems to delay biofilm development[262].

Good quality drinking water is important for health and production. Water entering the farm is often of excellent quality, but it deteriorates on the farm bacteriologically and chemically. The Dutch farmers organisation ZLTO has developed checklists for farmers to monitor and improve water quality for **pigs**[251] and **dairy cattle**[252]. Inadequate water quality or quantity may adversely affect **milk** yield and fertility. Water can be filtered. Flocculants can be used to filter invisible particles. Deferrization and de-ironing can be done via aeration or ionisation and this costs 6,000-9,000 euros. Finally, water can be disinfected by electrolysis, allowing pathogens to be filtered. Examples of such systems are the OxAqua (3,250 euros) and Watter[248]. Farmer Wijnen (NL) suggests using apple cider vinegar in drinking water of **poultry** to reduce AB use[20].

Since 2016 **broiler** farmer Boon (NL) has been warming up the birds' drinking water so as to reduce condensation of water on cold pipelines. In **veal** farming, it is already common to warm up drinking water before it enters the barn. In broilers it improves litter quality and foot sole lesions. Intestinal health and technical results (growth and meat quality) have also improved. The system costs 3500 euros, with a return of investment (ROI) within 2 batches. Each round costs 250 euros on gas. Boon expects the payback time for other farms to be less than 1 year. Heating drinking water improves animal welfare and it is also more pleasant to work in a dry barn[249].

Technical guides for water quality on **poultry** and **rabbit** farms are available, but they are not always effective. In order to sensitize farmers, a qualitative study was conducted with 11 rabbit farmers and 7 broilers farmers in France, to improve understanding of different attitudes to water management practices. Most farmers define water quality imprecisely. The farmers' level of knowledge and awareness are important. Farmers need more personal advice, encouragement and guidance. More suitable support measures are being developed[275].

A 2-page fact sheet is available in French to emphasise the importance to check water quality to improve **chick** starting[504]. AQUAPROTECT is an audit Excel Tool in French, for **poultry** advisors, to check in 30 minutes the main points of the water distribution system in broiler farms and to formulate recommendations to improve water management on the farm[509].

Water disinfectant treatment for **pigs, poultry and rabbits** may affect the stability of some AB in the water and their stability is multifactorial and complex[507].

Strategies and mechanisms for combating AB-resistant bacteria (ARB) and AB-resistant genes (ARGs) in wastewater were reviewed. High amounts of AB residue in wastewater promote selection of ARB and ARGs which find their way into natural environments. It is important to (1) study innovative strategies in large scale and over a long time, (2) do risk assessments to know the prevalence of ARB/ARGs and their risks to human health, and (3) understand and control the various treatment mechanisms[425]. Treated wastewater may be used as an alternative water supply, e.g. in agricultural irrigation. However, concerns exist over emerging contaminants such as ARB and ARGs. Case studies are presented of two countries that have already practiced water reuse. Our data suggest that wastewater treatment plants which are able to achieve at least 8-log reduction in microbiological contaminants may suffice as appropriate intervention barriers for ARB dissemination to the environment. The precautionary principle should be applied to implement appropriate intervention strategies and best management practices that minimize the impacts and concerns arising from the reuse of treated wastewater in agriculture[444].

5.5. Feed and gut health

Highlights

- Gut health is a vital driver of animal performance.
- Research is diversified due to the numerous health and production challenges.
- Colostrum is linked with a reduced disease incidence, reduced antibiotic (AB) use and a better welfare.
- Research in feed additives and supplements is rapidly evolving.
- Feed composition can improve gut health if it is carefully planned.
- Feed management can lead to an optimal productive performance, even without AB use, especially in combination with other measures (e.g. biosecurity measures).

What is feed or gut health?

Gut health is universally viewed as a vital driver of animal performance. With a healthy gut, an animal can perform its physiological functions (digestion and absorption of nutrients, establish a normal and stable intestinal microbiota) and withstand stressors (effective immune status, healthy tissues). A healthy gut is the animal's first line of defence, not only against disease-causing organisms, but also against dietary challenges that could negatively impact the pig's productive performance. The past two decades, a higher awareness of the impact of good livestock management profitability and the importance on disease prevention, as well as its role as a means to reduce AB use has enhanced the interest of the sector towards gut health. As a result, concepts related to gut health (e.g. dysbiosis) have been described, and increasing challenges (e.g. antimicrobial resistance, intensification of feed production) have led to the need for updated feed management strategies and the introduction of novel products (e.g. prebiotics) to the market as dietary supplements. Accordingly, research has diversified to meet the specific challenges faced when trying to set and keep a good gut health condition. Four categories address the main topics that dominate the gut health agenda: early feeding, feed composition, feed management and feed additives and supplements

Why is gut health relevant to reduce antibiotics?

Since the ban on the use of growth-promoting ABs in animal feed, dysbiosis has emerged as a major problem in intensive animal production. Dysbiosis or dysbacteriosis is defined as a shift in the intestinal microbiota composition resulting in an imbalance between beneficial and harmful bacteria. In response, the animal industry is actively promoting AB alternatives that show at least some of the benefits of AB (growth promotion, health protection). These alternatives range from several feed additives and supplements to the provision of colostrum and/or commensal adult intestinal microorganisms in newborn animals (competitive exclusion).

Some of these alternatives involve some form of a biological agent capable of either specifically inhibiting foodborne pathogens and/or function in a more broad-spectrum antimicrobial way while others (such as prebiotics) perform more indirectly. It is no secret that when disbalanced, the microflora can have a big impact on the digestibility of nutrients causing energy losses which will not be available for growth. Concepts such as competitive exclusion work by quickly inducing the formation of a diverse yet stable intestinal microbial flora and subsequently preventing pathogens colonizing the gut. In the absence of antimicrobial pressure, competitive exclusion products reduced the faecal excretion and transmission of AB resistant bacteria in animals. Likewise, colostrum, through its composition and transfer of passive immunity to the newborn mammalian livestock species, can improve survival rates, improve health and increase daily weight gain. Thus, the provision of a sufficient amount of good quality colostrum supports the rational use of ABs.

Alternatives strategies to reduce AMU focusing on feed/gut health

This next section will summarise examples from the animal sector divided by the main topic of interest.

Early feeding

This section could not start without focusing on colostrum and its multiple benefits. A UK award-winning industry campaign titled "*#ColostrumisGold*" has been launched online from RUMA (RUMA = Responsible Use of Medicines in Agriculture Alliance, https://www.ruma.org.uk/)[1]. Its mission is to communicate the benefits of colostrum and its role in improving welfare, reducing disease and reducing AB use in **cattle (dairy and beef), sheep and pigs**. As the website states: "Colostrum is chock-full of antibodies, energy and essential nutrients that can benefit the newborn animal." The website includes documents with Tech tools & Tips; selections of AB use case studies, showing implemented management changes on-farm that have reduced, refined or replaced AB use.

Acknowledging the importance of providing pathogen-free colostrum milk to newborn **calves**, the Flanders research center for agriculture and food (ILVO) studied whether a method to avoid infecting calves with *Mycobacterium avium* subspecies *paratuberculosis* (MAP) via contaminated colostrum could be developed[46]. When using a curdling process (analogous to making cheese) on site, they managed to eliminate 90% of the MAP germs. When an externally-located centrifugation treatment was used, the elimination rate increased to 98%. Both methods keep the required amounts of life-sustaining bioactive proteins while the MAP-related intestinal infections were strongly reduced. It appears that when applying this easy technique, farmers whose cattle have a limited infection can treat their colostrum on the farm. For heavier infections external treatment is recommended.

Group A bovine rotavirus (BRV) is the major cause of neonatal **calf** diarrhea worldwide. As a preventive strategy, an Argentinian study gave milk supplemented with rotavirus immune colostrum for the first 14 days of life. All calves received control colostrum prior to gut closure followed by the milk supplemented with immune colostrum, twice a day, for 14 days. Calves received milk supplemented with 0.8% immune colostrum (G1) or milk supplemented with 0.4% immune colostrum (G2). Calves were inoculated with rotavirus at 2 days of age. After challenge, all calves in G1 and G2 were fully protected against diarrhoea and only 1 out of 5 calves in G1 shed virus asymptomatically. Thus, this supplementation induced high protection rates against rotavirus diarrhoea in **calves** during the period of peak susceptibility to infection and a positive effect of the calves' immune responses[307]. The duration of the supplemented milk feeding were key factors to obtain high protection during the period of peak susceptibility to diarrhoea.

The effects of butyric acid supplementation in acidified milk (AM) on the digestive function of **calves** and on weaning stress were investigated *in vivo* by a Chinese study using 36 Holstein calves[406]. The addition of butyric acid to AM can reduce the rate of diarrhoea and weaning stress. Moreover, by improving the metabolic and physical development of the gastrointestinal tract it improved the overall digestibility.

Another Chinese study was set up to assess the effects of different sources of milk on growth performance, serum metabolism, immunity, and intestinal development of calves, 84 Holstein male neonatal **calves** were assigned to one of the following four treatment groups: those that received bunk tank milk (BTM), untreated waste milk (UWM), pasteurized waste milk (PWM), and acidified waste milk (AWM) for 21 days[407]. Bunk tank milk was found to be the best choice for calf raising compared to waste milk. Nonetheless, the provision of either pasteurized or acidified waste milk is an acceptable labor-saving and diarrhoea-preventing feed for young calves.

Focusing on the acidification of milk, an Australian study dealt with the acidification of milk that is then fed to **calves**[408]. Milk contaminated with bacterial pathogens (*Mycoplasma bovis* and *Salmonella enterica* ssp. *enterica serovar Dublin*) was fed to calves and then a commercially available acidification agent was used in the treatment group. The objectives of this study were to determine the growth of *M. bovis* and *Salmonella Dublin* in milk, and to evaluate the efficacy of milk acidification using this acidification agent. Milk acidification was effective at eliminating viable *M. bovis* and *Salmonella Dublin* organisms in milk when the appropriate pH and exposure time were maintained.

Feed additives and supplements

Research in feed additives and supplements is rapidly evolving, aspiring to produce local and systemic health benefits on a par with antimicrobials[29,324,390]. The potential of probiotics to positively affect animal health and inhibit pathogens has been demonstrated by numerous studies, yet experimental evidence suggests that probiotics' successes are modest, conditional, strain dependent, and transient. Phytochemicals, another promising alternative to antimicrobials, have been shown to stimulate feed intake and show antimicrobial, coccidiostatic and anthelmintic effects. However, little attention has been drawn to any safety concerns regarding the application of phytobiotics in livestock. Species-specific sections are provided below to go through the latest news over feed additives and supplements:

Pigs

A number of feed additives are marketed to assist in boosting the **pigs'** immune system, regulate gut microbiota, and reduce negative impacts of weaning and other environmental challenges[311,485]. Feed additives that have been used in pigs include acidifiers, zinc and copper, essential oils, herbs and spices, some types of prebiotics, bacteriophages, anti-microbial peptides, prebiotics, direct-fed microbials, yeast products, immunoglobulin, nucleotides and plant extracts. Inclusion of pharmacological levels of zinc and copper, certain acidifiers and several plant extracts have been reported to result in improved pig performance or improved immune function of pigs[311]. It is also possible that use of prebiotics, direct-fed microbials, yeast and nucleotides may have positive impacts on pig performance, but results have been less consistent and there is a need for more research in this area. One approach to evaluate the effectiveness of feed additives *in vivo* is to use an appropriate disease challenge model[485].

Strategies aiming at stimulating natural host defenses through the use of substances able to modulate immune functions have gained increasing interest in animal research, and different bioactive components *a priori* sharing those properties have been the subject of several *in vivo* nutritional investigations in **pigs**[383]. Immunomodulators such as yeast derivates (b-glucans and mannans), plant extracts and spray-dried animal plasma have been studied with animal plasma being the one with the most promising results. However, the lack of standardization of extracts and the heterogeneity of piglet-rearing conditions limit the interpretation of the results so far.

Another review paper focused on the use of organic acids as feed additives on early weaned **piglets** (3-4 weeks age). Early weaned piglets are exposed to stress with a reduced feed intake, and little or no weight gain. This post weaning lag period is due to a limited digestive and absorptive capacity due to insufficient production of hydrochloric acid, pancreatic enzymes and sudden changes in feed consistency and intake. Lowering dietary pH by using organic acids was found to overcome these problems[476]. In addition, organic acids enhance apparent total tract digestibility and improve growth performance. Apart from that, organic acids have shown bacteriostatic and bactericidal activities. Lactic acid has been reported to reduce gastric pH and delay the multiplication of an *enterotoxigenic E. coli*.

Oregano essential oil provides support for animal health and performance due to its active compounds (carvacrol and thymol) that have been shown to have both antimicrobial and antioxidant functions. A British research group studied the effect of the addition of natural oregano essential oil in the diet of breeding **sows** and the beneficial results on **piglets** before and after weaning[96]. The research was carried out in a commercial pig unit in the UK and involved 62 multiparous sows. The conclusion was that the inclusion of oregano essential oil helped maintain the body condition score of suckling sows, increased weight gain during the first week of life of piglets, decreased pre-weaning mortality and significantly decreased AB use.

With the rapidly increasing knowledge of the role of the gut microbiome in diverse aspects of human and veterinary health, antibody-type drug-mediated methodology to specifically interfere with the microbiome or host factors in the gut is needed. Oral antibodies that interfere with gastrointestinal targets and can be manufactured at scale are needed. A Belgian paper shows that a single-gene-encoded monomeric immunoglobulin A (IgA)-like antibody can prevent infection by enterotoxigenic *Escherichia coli* (F4-ETEC) in **piglets**[341]. This antibody can be produced in soybean seeds or secreted from the yeast *Pichia pastoris*, freeze- or spray-dried and orally delivered within food.

Addressing a major public health concern, a Belgian *in vivo* study examined the effect of three intervention strategies on *Salmonella Typhimurium* transmission in **pigs**[145]. The first intervention was feed supplemented with coated calcium-butyrate, the second comprised oral vaccination with a double-attenuated *Salmonella Typhimurium* strain, and the third was acidification of drinking water with a mixture of organic acids. Both feed supplementation with coated calcium-butyrate and vaccination with an attenuated vaccine decreased

Salmonella Typhimurium transmission in pigs. Further studies are needed to assess the practical issues related to the implementation of these interventions. For example, more data are needed to determine the best age groups and treatment regimens for the coated calcium-butyrate and to learn how to overcome the problem of Salmonella-specific antibodies in vaccinated pigs.

A Brazilian study aimed to evaluate the effects of dietary Brazilian red pepper essential oil and an antimicrobial agent on **weanling pig** growth performance, diarrhea, pH of the digestive content, small intestine cells and intestine microbial counts[285]. Ninety weanling castrated male pigs were given five treatments: a diet supplemented with 0 (negative control), 500, 1000, and 1500 mg/kg Brazilian red pepper essential oil and a diet supplemented with 120 mg/kg chlorohydroxyquinoline (AB). Treatments had no effect on growth performance, diarrhoea occurrence, pH of the digestive content, gut mucosa and intestinal microbial counts of weanling pigs. However, pigs fed the diet containing 500 mg/kg essential oil had thicker gut mucosa than those fed diets containing the antibiotic or 1000 and 1500 mg/ kg essential oil. Overall, Brazilian red pepper essential oil and the AB are of limited benefit for enhancing the growth of weanling pigs.

The effect of allicin on health and growth performance of weanling (21 days old) **piglets** was investigated in China[288]. Two hundred and twenty-five piglets were weaned and allocated into five groups. Piglets in the control group were fed diets supplemented with ABs. In the treatment groups pigs were fed diets without ABs, but supplemented with an allicin product (25% pure allicin oil) with 0.10 g/kg, 0.15 g/kg, 0.20 g/kg and 0.25 g/kg in the diet, respectively. After 28 days, the average daily weight gain increased as the level of dietary allicin increased; whereas feed gain ratio, the incidence of diarrhoea in the treatment piglets decreased (especially female piglets) and the number of flies on the surface of the faeces decrease with increased allicin. In conclusion, diets with allicin may improve growth performance, reduce the incidence of diarrhoea and improve their local environmental conditions due to a lower number of flies.

The effects of hop (*Humulus lupulus*) beta-acids on several **pig** production parameters were compared with diets containing colistin in a Brazilian study[293]. The parameters were body weight (BW), average feed intake (ADFI), average daily gain (ADG) and gain:feed ratio (G:F). Two hundred weaned **piglets** (21 days old) fed diets supplemented with 0 (negative control), 120, 240, or 360 mg/kg hop beta-acids, or with 40 mg/kg colistin (AB control). Increasing dietary levels of hop beta-acids improved BW, ADG, G:F and digestibility in weanling pigs. The colistin treatment also improved BW, ADG, and G:F compared to the negative control. In conclusion, dietary hop beta-acids concentrations up to 360 mg/kg improved weanling pig growth rate by affecting the efficiency of feed utilization, at a similar rate as observed when administering colistin.

Synthetic porcine beta-defensin-2 (pBD-2) was tested as an alternative to antimicrobial growth-promoters in pig production in China[294]. Thirty **weaned piglets** (21 days old) were challenged with *Escherichia coli*, and orally dosed with either sterile water (CON), pBD-2 (BD) or neomycin sulphate (NS) twice daily for 21 days. PBD-2 has antimicrobial activity in piglets, and it can improve growth performance, reduce inflammatory cytokine expression and affect intestinal morphological indices in the same way as probiotics. Moreover, pBD-2 has a more positive effect on intestinal morphological indices and intestinal probiotics of piglets than does NS. Therefore, the present results suggest that pBD-2 may be a suitable replacement for NS in piglets.

To prevent and control enterocolitis in **pigs,** various non-AB products have been launched. For example, a feed bioadditive product based on polyculture with selected strains of lactic acid bacteria that produce lactic acid and some bacteriocins is available in Romania[385]. It works by populating the digestive tract with these commensal bacteria and prevents the growth of pathogenic bacteria.

In a lay article from Australia, **pig** farm management reflections on how to best treat *E. coli* without using AB and/or zinc oxide were discussed[501]. After years of veterinary practice, the author proposed the use of the following action plan to achieve optimal health and performance at four weeks post weaning: a) attention to detail, b) all-in-all-out system, c) hygiene-focused, d) weaner diet consisting of less than 18.5% protein. Acknowledging the specific differences between countries/continents, different mixes of feed additives were proposed: a European programme consisting of coated butyric, formic and citric acid and *Bacillus* PB6, a Korean programme consisting of Algal immune stimulants etc.. With regards to zinc oxide, the advice is to remove zinc oxide gradually because if management is not as good as expected, and you have a very pathogenic *E. coli*, then *E.coli* may triumph over lack of zinc oxide.

Poultry

Maintaining an optimal poultry gut health is a prerequisite for reaching the high productivity standards that are sought for this species. Banning preventive in-feed antibiotics has increased risks for outbreaks of necrotic enteritis, predisposing from coccidiosis and overloads of nutrients in the intestine in **broilers**. Different dietary strategies can be used to fight coccidiosis. Some products act against certain species (e.g. *Eimeria*), such as essential oils and herbal extracts. Other products beneficially modulate the immune status of the chicken, whereas prebiotics and probiotics improve microflora to reduce the chance of secondary infections[157]. Phytogenic feed additives (PFAs) are used to tackle necrotic enteritis either *via* feed or *via* water[214]. Drinking water application of PFAs is flexible and quick and is increasing in popularity. Application can take place in combination with other additives such as organic acids, probiotics, or vaccines. An improved broiler gut quality and function results in a reduced disease incidence and less treatment costs.

In **layers**, preventive nutrition strategies that support gut performance can offer a cost effective alternative to AB use, particularly around peak production[213]. In fact, unsaleable eggs and the challenge of drug-resistant bacteria can make AB application more expensive than non-AB alternatives. In Hungary, a commercial layer farm successfully reduced its AB use by applying several preventive nutritional strategies using commercially available feed additives[213].

Since the European Union enforced the ban on AB growth promoters in 2006, inulin is one of the most commonly used and most effective probiotic additives[420]. The mechanism of inulin interactions with the avian body is complex, multidirectional and not fully understood.

Despite a number of unresolved issues, many authors have demonstrated the positive impact of inulin on the host organism. The reports on inulin effects on the body and performance of **poultry** are often contradictory, as the effectiveness of this prebiotic is strongly dependent on the type and dose used, and the duration of its administration.

A recent review focused on the use of pumpkin seed extract and its effects on **poultry** health and nutrition[418]. Pumpkin seed extract is reportedly useful for immunomodulation, reproductive health, treating a wide range of disease conditions and for the metabolism of accumulated fats[418]. Studies have also shown that pumpkin seeds are a valuable source of protein and fat. Their complexity and extent of bioactivity offer sustainable prospects for natural control of pathogenic or parasitic organisms making a good case for the use of pumpkin seed extracts to improve gut health.

A Chinese study investigated the effects of protected essential oils (P) and organic acids on **poultry** feeding[419]. Product supplementation improved spleen index, villus height and crypt depth of the jejunum at 42 days when compared with a control diet. In addition, secretory immunoglobulin A level of ileal mucosa and trypsin and chymotrypsin activities of the intestinal tract were higher in the P treatment. Bacterial sequence analysis of the intestinal tract revealed that protected essential oils and the supplementation with organic acids changed gut microflora mainly in *Lactobacillus*. These data suggested that dietary supplementation with organic acids and essential oils could be used in the poultry industry as an AB growth promoter alternative.

In **poultry**, zinc is mostly provided by in-feed supplementation, mainly as zinc oxide or zinc sulphate. A Belgian study was set up to show whether the supplementation of zinc with amino acids (organic form of zinc) has an effect on gut health of **broilers** compared to zinc oxide or zinc sulphate (inorganic form of zinc)[368]. The best results were attained when supplementing feed with an organic zinc-amino acid complex, especially in stressful periods. In particular, zinc supplied in feed as amino acid complex is more readily absorbed, potentially conferring a protective effect on villus epithelial cells in the starter phase.

An experiment using **broiler chickens** was conducted in Moldova to assess the influence of a commercially-available feed additive (a product containing *Bacillus licheniformis* and *Bacillus subtilis*, betaine, vitamins and microelements)[386]. The product was administered in the drinking water (1 g/litre) from the age of 9 to 14 days (experimental group). Performance were measured weekly and blood samples were collected for biochemical and haematological analyses. A reduced mortality (-3.5%) and a higher body weight (+50 gram) was seen in the experimental group as compared to the control group. Apart from that, higher levels of erythrocytes, haemoglobin, total protein and glucose in blood serum were seen in the experimental group.

Cattle

A review on non-AB interventions in the form of products or management practices that could potentially reduce the need for AB in **beef** and **veal** animals living under intensive production conditions was conducted[347]. The objectives were 3-fold: first, to examine and describe the range and nature of research on non-AB approaches that may ultimately reduce the need for medically important ABs to prevent, control or treat illnesses in beef and veal production; second, to identify areas where systematic reviews could summarize the effect of specific non-AB approaches within the broader topic area; and third, to identify knowledge gaps where additional primary research might provide valuable insights into the effectiveness of different specific non-AB approaches. The four most frequent interventions were: non-AB feed additives, vaccinations, breed type and feed type.

Feed composition

When carefully selected and planned, feed composition can be successfully used to improve gut health and as a result reduce the need for antimicrobials[481].

The size of feed particles and its effect on the absorption of nutrients was reviewed by Kiarie and Mills[309] Finer feed particles were associated with a higher absorption of nutrients by allowing better contact with digestive enzymes and enhancing thus the performance of the animal. Yet, very fine particles negatively affect gut health leading to stomach ulceration in **pigs** and gizzard dysfunction in **poultry**. In general, coarse particles increases stomach and hindgut acidification, which may be beneficial in controlling overgrowth of gut bacteria, such as *Salmonella spp.* and *E. coli*. However, since most commercial pig/poultry diets are subject to heating to reduce feed-borne bacteria (e.g. *Salmonella spp.*) reduction of feed-particle size is inevitable. However, achieving high nutritional quality and processing at high temperatures does not favour high nutrient availability and stability of heat-sensitive enzymes. Therefore, feed processing must be balanced for maximum nutrient utilization, feed hygiene status, stability of enzymes post-processing and impact on gut health.

The utilization of dietary fibre (DF) by **pigs** and its effects on gut physiological functions, microbiota and health was reviewed[481]. There seem to be both negative and positive effects of different fibres in swine diets. More specifically, while DF seems to lower nutrient digestibility in swine, the fermentation of DF in the gut may positively modulate the gut environment and potentially favour 'beneficial bacteria', thereby improving gut health of pigs. The impact of dietary fibre on pig nutrition and health is determined by the fibre properties, and may differ considerably between fibre sources[480]. When carefully selected and planned, utilizing dietary fibre from different sources can be used to improve gut health.

The benefits of using rye as animal feed are discussed in an interview with a German farmer who cultivates rye[384]. In his view, rye needs less fertilizers and he noticed a positive effect on the health and welfare of his animals. Rye has a large amount of carbohydrates that are not digested in the small intestine. As a result the carbohydrates feed the microorganisms in the small intestine and microflora. This produces butyric acid, which has a positive influence on animal behaviour during fattening. Butyric acid is also very effective in killing bacteria such as *Salmonella spp.* However, as discussed in the review paper of Lindberg, corn and soybean meal are still the main staples in the diet for **pigs** and **poultry**, providing most of the energy and nutrients needed. It is argued that although other cereals, such as wheat, and by-products, such as rice bran and distiller's grains, are used as alternative feedstuffs in parts of the world the quantities available are not sufficient to replace corn and soybean meal in the global **pig** and **poultry** industry[480].

Feed management

The optimization of feed management seems to have boosted the colostrum production of a Northern Ireland **sheep** producer leading to a dramatic cut in AB treatment for watery mouth in newborn lambs[10]. The interviewed farmer believes that a focus on **ewe** health and feed management has led to a visibly-improved colostrum quality. The big reduction in the prevalence of the watery mouth that was noticed was followed by a big reduction in AB use.

A Dutch meta-analysis review study showed that post-hatch food deprivation or post-hatch food and water deprivation (PHFWD) for approximately 24 hours can lead to significantly lower body weights compared to early-fed **chickens** up to six weeks of age[85]. Body weights and food intake were reduced more if the food and water deprivation lasted longer. PHFWD also has negative effects on the development of the liver and pancreas, and delays the development of duodenum, jejunum and ileum. These latter effects mainly appear to be short-term. The authors concluded that 48 hours (>=36±60 hours) PHFWD leads to lower body weights and higher total mortality in chickens up to six weeks of age, the latter also suggested compromised chicken welfare.

Neonatal **piglet** diarrhoea is a major cause of pre-weaning mortality, resulting in significant economic loss for swine producers. A retrospective study on the etiological diagnoses of diarrhoea in neonatal **piglets** in Ontario, Canada, between 2001 and 2010 was set up[483]. The relative importance of different diseases contributing to neonatal diarrhoea in piglets appears to be changing, possibly because of changes in husbandry and management practices, advances in diagnostic techniques, and/or the emergence of new diseases. This study identified several current pathogens involved in neonatal diarrhoea for Ontario pig farms. *Clostridium difficile* appears to be an emerging pathogen, and *enterotoxigenic E. coli* (ETEC) and rotavirus remain pathogens of concern for neonatal diarrhoea in piglets. Further research in the diagnostic method of these pathogens may be useful in improving the diagnostic rate for gastro-intestinal disorders. The data suggested that *C. perfringens* type A may be an important pathogen for neonatal diarrhoea in piglets, but the current lack of specific diagnostic criteria for this pathogen made it difficult to determine its significance.

A US study was done to measure the effects of quaternary benzo(c)phenanthridine alkaloids (QBAs) against *Salmonella spp* and determine subsequent effects on growth performance, *Salmonella* shedding, and gastrointestinal tract integrity in **pigs** inoculated with *Salmonella Typhimurium*[289]*.* Inoculated pigs were placed in 4 groups receiving a control (not-supplemented) diet or a diet supplemented with 1.5 or 0.75 g of QBAs/1,000 kg of feed, or 59.4 g of chlortetracycline/1,000 kg of feed. Both diets containing QBAs decreased the shedding of *Salmonella spp*.. Forty days after inoculation, the shedding was lower for pigs fed diets containing QBAs or chlortetracycline. Growth performance was similar for pigs fed diets containing QBA or chlortetracycline. Gastrointestinal tract integrity improved in pigs fed the diet containing 1.5 g of QBAs/1,000 kg of feed.

A US innovation program called “Seed, Feed and Weed” is an alternative approach to **poultry** gut health, similar to the competitive exclusion concept[314]. This feed management program includes three separate steps: “seeding” the gut with favourable organisms, "feeding" the favourable organisms and "weeding" out the unfavourable organisms. This involves applying a probiotic/competitive exclusion product as soon after hatch as possible. Adding a suitable organic acid via the drinking water helps the favourable organisms to increase in concentration in the small intestine. When paired with effective biosecurity measures, this approach allows the animals to achieve optimal productive performance, even without AB use.

A Spanish study tested two strategies (feeding management, higher feeder space) to improve the growth rate of the slow-growing **pigs** and to increase the batch's homogeneity at slaughter[470]. Under commercial conditions and with the genetic lines used in this study, it was concluded that higher feeder space availability may improve both BW and ADG in the growing and finishing periods. **Pigs** allotted more feeder space have a lower number of wounds and tend to have lower BW variability during the growing and finishing phases of production, respectively. Regarding feeding management, the results suggest that the light piglets, subjected to a specific feeding strategy at the start of the growing period, increase their growth rate and partially catch up with their bigger/heavier counterparts, leading to significantly decrease the variability of the population at slaughter.

Another Spanish study aimed to evaluate the impact of an alternative feeding program based on unmedicated diets formulated with fibrous by-products and functional feed ingredients on performance and faecal microbiota of young **pigs** compared to a common weaner diet supplemented with AB[469]. The alternative feeding program could facilitate gut development of young piglets, which at the end of the nursery period presented a faecal microbiota more similar to that found in fattening animals. Moreover, piglets fed the unmedicated diets showed a trend to reduce the course of diarrhoea immediately after weaning. The alternative feeding program showed, however, a reduced growth efficiency during the nursery period that needs to be discussed in the frame of the costs-benefits analysis of reducing AB.

A Dutch lay article presented five simple feed management tips to avoid heat stress in **cattle**[253]. Tip 1: dairy farmers should not feed at the hottest time of the day. Tip 2: To keep the feed tasty and fresh, it is advisable to provide fresh feed several times a day (at least twice) and to (mechanically) ventilate the barn well over the feed alley. Tip 3: In order to prevent rumen acidification, which occurs more often in heat, a buffer can be added in consultation with the feed supplier in order to maintain the acidity level in the rumen. Tip 4: Bring the cows inside at the hottest time of the day and make sure there is unlimited water available. Tip 5: Clean cubicles are also necessary in case of heat. Bacteria in cubicles thrive in the heat and grow exponentially (e.g. risk of *Klebsiella* mastitis).

5.6. Precision Livestock Farming (PLF) and early disease detection

Highlights

- PLF is an approach to managing livestock using automated and continuous data in real time that aids farmer/advisor decision making.
- PLF uses data collected from wearable sensors like collars and ear tags, camera or microphone systems installed on farm infrastructure, climatic sensors and robotics. These large datasets can then be processed and analysed quickly and easily by computers through artificial intelligence (AI), machine learning and the Internet-of-Things, amongst others.
- The data is distilled into simple actions and alerts on mobile devices that farmers and advisors can respond to.
- PLF has been used for prediction and early disease detection in **cattle** with mastitis or lameness, also for the automation of responses to changing climatic conditions in **poultry** sheds and to inform farmers about stress levels in **pigs**.

What is Precision Livestock Farming?

Precision Livestock Farming (PLF) is an approach to the management of livestock by semi to fully continuous, automated and real-time monitoring of production, fertility, health and the welfare of individual animals and the environmental impact they have. The technologies used in PLF aim to capture and analyse data 24/7 and produce warnings for farmers and other key stakeholders when there is an issue or something wrong with the animal. Monitoring and capture of data can be visual using cameras, through sound using microphones, and e.g. by movement sensors worn on the animal or stationed in their near environment.

PLF is also often referred to as Smart Farming and has applications across all farming sectors including non-livestock. It includes novel technologies that can be used at an individual animal level, such as rumen boluses and accelerometer ear tags on **dairy cows** or movement sensors on **sheep**. It also includes infrastructure technology, such as cameras in sheds using machine learning to monitor the activity of **broiler** chickens or robotics during harvesting or for land-based work. PLF also incorporates decision support systems for farmers, such as phone Apps and data analysis programmes.

Why use PLF to reduce AMR?

PLF aids farmer decision making, helping farmers to detect disease in individual animals earlier, rather than relying on human labour, thereby reducing the need for ABs. PLF can help farmers monitor an animal's performance and intervene with non-AB treatments. Promoting the use of these types of management tools will be very helpful for farmers looking at ways to help them reduce disease in their herds and flocks. It is therefore directly relevant to the DISARM network.

Examples of Precision Livestock Farming

PLF is being used across species round the world. This next section will summarise examples from the **dairy** sector followed by the **pig** and **poultry** sectors.

Dairy

For **dairy** cattle, a review article has compiled different aspects of smart dairy farming, setting out a state-of-the-art framework that can assist farmers to increase milk yield by using the latest technologies[226]. These technologies give early health alerts from individual monitoring of activity, rumination rate, lying time, eating time and temperature, and are useful in monitoring at a herd level to optimise working practice and minimise nutritional stress. These technologies can decrease the factors negatively affecting milk production and increase those positively affecting production with minimal resources, such as ABs. Further to this, a UK report detailing the differences between top and bottom performing farms poses some key questions to understand the variation in performance and animal health between farms[172].

PLF in **dairy** is often focused on mastitis. Typical diagnostic methods are based on somatic cell count {SCC) and plate-culture techniques. These methods are not quick or real-time, which is needed to speed up the choice of treatment. Robotic milkers are capable of taking real-time measurements based on milk conductivity but a more novel development are Biosensors - tools that can convert the presence of biological particles into an electric signal. Together with microfluidics, biosensors can be used in the development of automated and portable diagnostic devices. A review from Portugal describes current approaches for mastitis diagnosis and the latest outcomes in biosensors and lab-on-chip devices with the potential to become real alternatives to standard practices[87].

It is a common misconception that PLF has to be high tech! PLF can be done by using a simple Excel spreadsheet-based tool developed by the Agriculture and Horticulture Development Board (AHDB) and the University of Nottingham in the UK. A farmer can analyse herd-level data easily and receive a report on the pattern of mastitis on their farm[179]. This tool helps farmers to tackle mastitis in a systematic and evidence-based way. The tool allows the farmer to input data from bulk milk tank readings, SCC data and mastitis records to evaluate which bacteria and infection patterns are present in the herd, and which measures can be taken to control outbreaks and reduce the mastitis rate. The tool identifies problem areas and potential risks to udder health and gives farmers and vets a way of tracking progress in the herd.

A similar PLF application from Romania demonstrates how integrated software programmes can pull together and analyse vast amounts of data for **dairy** farmers[278]. A management system database - SGBD - is a software programme for production and reproduction data management on dairy farms, as well as for accounts and genetic breeding of bovine herds. SGBD integrates multiple programmes, which allows the farmer to access large amounts of information for health and business planning. It provides a fast and a cost-effective way of exploring complex data and gives farmers quick access to dates and cow numbers.

However, the adoption of technology in European **dairy** farming lags behind compared to other farming sectors; less than 20% of all dairy farmers use fertility management tools and less than 10% use feed and health monitoring systems[232]. A Dutch-led project called Happy Cow aims to encourage technology uptake in dairy farming by combining advanced big data analysis with machine learning technologies[232]. Happy Cow aims to improve dairy farm productivity through 3D cow activity sensing and cloud machine learning. In turn it can detect health issues at an early stage, such as clinical milk fever or rumen acidosis, and help farmers make evidence-based decisions early on, such as those with hard-to-detect lameness, thus safeguarding animal health and increasing the farm's productivity.

One study from Canada used models designed to predict which **cows** are likely to become ill based upon measures of the cows' feeding and competitive behaviours before calving[400]. The models had high sensitivity, specificity, and predictive values for both cows that had previously calved and for those calving for the first time. Behaviours in the feed area before calving can predict cows at risk of becoming sick in the weeks after calving.

Also, from Canada, a study using data loggers and electronic feeding systems identified changes in feeding, social and lying behaviour, which contributed to identification of **cows** at risk of metritis[413]. During the 2 weeks before calving, cows later diagnosed with metritis had reduced lying time and fewer lying bouts compared with healthy cows. In the 3 days before clinical diagnosis, cows that developed metritis ate less, consumed fewer meals, were replaced more often at the feed bunk, and had fewer lying bouts of longer duration compared with healthy cows. Using PLF to identify changes in feeding, social and lying behaviour could contribute to detect cows at risk of metritis and thereby allow farmers to intervene with alternative treatments to ABs before the condition worsens.

Timely lameness detection is key to successfully reducing lameness in **dairy** cattle – a major health and welfare concern that ultimately affects milk productivity. To tackle this, an end-to-end Internet of Things application that leverages advanced machine learning and data analytics techniques to monitor cattle in real-time and identify lame cattle at an early stage has been developed in Ireland[399]. The proposed approach has been validated on a real-world smart dairy farm setup consisting of a herd of 150 cows in Waterford, Ireland. The detected lameness anomalies are then sent to the farmer's mobile device. The results indicate that lameness can be detected 3 days before it can be visually detected by the farmer with an overall accuracy of 87%. This means that the animal can either be isolated or treated immediately, improving cure rates and reducing the need for excessive AB use.

The use of mobility sensors either on a cow's leg or from a collar around her neck have been used in multiple projects around the world; using machine learning to analyse the sensor data simple alerts can be programmed to be sent to farmers on a phone or computer to either, check on a cow or intervene with non-AB treatment, such as a foot trim early on[228].

Multi-sensor cow monitoring can help farmers prioritise their time and spend more time on sick cows[227]. A European project involving *Moonsyst* and *GenXsen* aims to increase cattle insemination rates by 10%, decrease time spent observing cows thus freeing up time for leisure for the farmer, and reduce medication costs by 10% all by using a smart rumen bolus and cow activity neck collar. The culmination of data from these 2 sensors and a cloud-based service to store and analyse the data allowed farmers to track and act upon comprehensive evidence rather than on observations alone.

A Belgium-Dutch field trial using '*Connecterra's Intelligent Dairy Assistant*' (IDA), is a further PLF example exploiting a novel Internet of Things management support system for dairy farms[397]. IDA uses sensor technology, cloud computing and artificial intelligence to support dairy farmers with insights on cow oestrus and health management. The IDA system uses feedback on historic data to improve its underlying models and farmers learn to use the data outputs from the system. The trial indicated that oestrus detection can be improved, and health monitoring can help to start early treatment and thereby reduce excessive AB use. The impact of this technology on milk production was inconclusive from this study.

Looking at the wider milk supply chain, there have been advances in using PLF concepts for remote milk quality. Central milk and dairy testing laboratories use InfraRed (IR) analyses instruments to analyse milk composition and quality[230]. However, it is a challenge to keep instruments calibrated, controlled and monitored throughout the dairy chain. A Dutch-Belgium-German collaboration is working on a quality assurance service of locally obtained milk and remote dairy composition analyses using sensors. Analytical instruments can be monitored remotely and validated by use of reference samples, calibration sets and software applications. If necessary, adjustments can be carried out remotely and in real time.

Pigs

Precision Livestock Farming in the pig sector is also well developed[396]. Outputs from sensors (e.g. activity measures with a camera or sound measures using microphones) are related to animal welfare and health indicators such as sow/weaner aggression or respiratory diseases. When sensor signals start to deviate from their expected values, alerts are sent to the farmer. In this way the farmer can take an immediate action before the detected change in animal response negatively affects production. These actions include solving technical problems, such as a blocked feeding line or adjusting control settings in the climatic controller. In most cases, a preventive medical treatment prevents the further spreading of respiratory diseases in the pen, and AB use can be reduced or even precluded. However, it is recommended that adequate training of farmers and the further integration of the PLF system in operational management systems is addressed.

A demonstration project in Flanders, Belgium, using the *SOMO Respiratory Distress Monitor* of SoundTalks in 10 commercial fattening pig houses, produced an automatic alarm when respiratory problems occurred[398]. The warnings of the SOMO-system were analysed against the observations of the farmer. In most cases (74%) the alert situation was confirmed by the farmers' inspection, and in 17% of the cases farmers started a medical treatment based on the alerts. At the time of the alert, the number of sick animals was still low and the behaviour (activity, feed intake) of the animals still normal in most cases (86%). It was confirmed by the farmers that the use of the SOMO system helped to reduce the amount of medication, because treatments were done in an early stage of infection with better cure rates.

Sound-based PLF techniques have significant advantages over other technologies, such as cameras. Besides the fact that microphones are contactless and relatively cheap, there is no need for a direct line of sight and large groups of animals can be monitored with a single sensor in a room[320], albeit the outputs are not specific to individuals in a group situation. A respiratory distress monitor trialled in Belgium automatically monitors the respiratory health status of groups of pigs. The study demonstrated that the tool works for the early detection of animal responses due to technical environmental issues (ventilation problems) and health issues in a wide range of different conditions in commercial European pig houses.

An exciting Internet-of-Things application in the pig sector is working to reduce boar taint across European pig production[234]. The project aims to provide the farmer with management information that enables continuous improvement. The project has created over 2000 pig records, involves 5 farms and pulls together data covering genetics, the farmer, the slaughterhouse, processor and retailer, right through to the end consumer. The goal is to develop early warning systems on several group-level daily data streams, report back boar taint presence to farmers and link with preventive measures. It is also hoped this PLF application will improve feed efficiency, animal welfare and lower the carbon footprint of the end product.

Poultry

Another sector that has embraced PLF is poultry. Through optimising production, transport and processing of poultry meat by automated environmental monitoring and control, as well as advanced data analyses, there is real-time data being fed back to the farmer on the health of their birds and any early signs of disease[237].

How can PLF help reduce the need for ABs?

PLF is allowing farmers and their advisors to use comprehensive data to inform their decision making so diseases can be detected earlier and sick animals can be treated and recover sooner, leading to better treatment outcomes and improved productivity. PLF also allows management of animals with non-AB interventions thus reducing AB use. PLF and the capabilities of machine learning and artificial intelligence has enabled complex datasets to be reduced to simple steps for farmers and advisors, avoiding the need for laborious and skilled analysis. Aggregate datasets also allow population level management to prevent and predict issues. The different ways of capturing data at the farm level - whether from wearable sensors, cameras or microphones - means there are many applications specific to different farming systems.

5.7. Breeding for resilience

Highlights

- Breeding techniques and technology have greatly improved in recent decades – from phenotypic selection to gene edited animals.
- Selecting livestock that have genetic traits that result in resilience to disease and resilience to changes in their environment can lead to a reduced need for antibiotics (AB).
- Different breeds within a species have variation in resilience to disease - understanding which breeds might be better for which system can lead to reduced AB use.
- Certain genes have been identified in **pig** and **poultry** species that confer resilience to major production diseases- breeding from animals with these genes or even using technology, such as gene editing to include these favourable traits in their genome has the potential to improve animal health and welfare.

What is breeding for resilience?

Resilience is an animal’s capacity to be minimally affected by, or to quickly recover from, challenges to their physical and mental states. Challenges may include disease, temperature stress, novel environments, human interactions and changes to social groups. An animal’s ability to cope under different conditions is in part determined by its genetics. Genetics plays an important role in determining animals’ susceptibility to disease and their response to other physical, environmental and social stressors. Selective breeding for favourable genetic traits can make them more common in future generations.

Why is it relevant to reducing ABs and the DISARM network?

Resilient herds and flocks experience very little disease, such as respiratory disease or diarrhoea, and therefore need very little AB use over their lives. Helping farmers and advisors breed animals with the genetic potential to be disease resilient is beneficial to reducing the need for ABs. Breeding for improved resilience fosters good health and wellbeing of livestock over future generations, benefiting overall farm performance and sustainability. Indicators for general resilience are currently being researched, but health-related traits like longevity and good growth rates can act as indicators for disease resilience, thereby promoting healthy livestock. Sharing best practice and innovation in this area is therefore directly relevant to the DISARM network.

What is interesting and worthwhile knowing about breeding for resilience?

First, we will summarise some general state-of-the-art resources on breeding for resilience and robustness and then move onto species-specific examples.

Kiplagat's *‘Genetic Improvement of Livestock’* presents issues pertaining to genetic improvement of livestock for production[223]. It covers basic population dynamics, quantitative genetics and molecular genetics, and their application in animal breeding. The use of specific gene information can help to increase rates of genetic improvement and open opportunities for using additive/non-additive genetic techniques in domestic species, provided this new technology is optimally used together with more ‘traditional' or ‘conventional' methods based on phenotypic information. A rational use of molecular methodologies in genetic improvement of milk production requires simultaneous selection of all genes affecting important traits in the population. Maximum benefit can be obtained when these techniques are used in conjunction with reproductive technologies, e.g. artificial insemination, and collection and production of in vitro embryos to accelerate genetic change.

A useful resource in the state-of-the-art database that is applicable across livestock species is the '*Low Input Breeds'* project report, entitled – *Developing integrated breeding and management to improve animal health, product quality and performance in organic and low input milk, meat and eggs*[225]. The breeding of **laying hens, pigs, sheep and cattle** was evaluated for ethical, economic and environmental impact. By developing and integrating (a) genotypes selected for performance, robustness and product quality traits, and (b) managing innovations to improve 'low input' systems (I.e. farming systems with little reliance on costly inputs, such as fertiliser, pesticides, machinery, complex feeding systems, medicines, etc.), this project made a significant contribution towards regionally-adapted breeding strategies, compatible with sustainable production, high product quality and organic principles. Hence, this is very relevant to helping advisors and farmers choose breeding strategies based on organic principles with low reliance on AB inputs.

Cattle

Dairy cows have very high metabolic demands and often have increased difficulties adapting to changing environments, which can lead to disease and poor health. This leads to increased replacement rates and frequent occurrence of diseases and consequent medicine use. An article from Italy called *'Control of bovine mastitis in the 21st century: immunize or tolerize?'* reviews how a good understanding of the immune system is crucial in managing mastitis in dairy cows[40].

Specific to cattle, an Italian research project using a multidisciplinary approach, compared innate immune responses, metabolic parameters, milk protein profiles and milk microbiota in **Holstein Friesian and Rendena cows** reared on the same farm and under the same management conditions[36]. Results showed that Holstein Friesian and Rendena cows have different metabolic traits. Mastitis markers were higher in Holstein Friesian milk. The microbiota biodiversity was lower in Rendena milk. The colostrum protein profile was markedly different in the two breeds. Mammary innate immune response patterns displayed breed-specific differences. The observations reported in this work present numerous factors that may provide more robust/rustic breeds with a higher resistance to disease and therefore less need for AB use.

In the UK a popular genetic ranking index exists for farmers, published by the national levy board as part of its genetic evaluation service, called '*The national Profitable Lifetime Index (£PLI)*'[171]. The £PLI is recommended for use by **all-year-round calving** UK farming operations. £PLI should be used as the initial screening tool in **bull** selection, then a deeper look within this group of animals for the traits that most need improving in your herd. The £PLI value represents the additional profit a high £PLI bull is expected to return from each of its milking daughters over her lifetime compared with an average bull of £0 PLI. High £PLI will result in cows with improved udder conformation and feet, better calving performance and reduced costs because of improved milk quality and fertility.

State-of-the-art from the other side of the globe includes a New Zealand review that looked at the breeding challenges from extensive outdoor grazing systems[224]. Although food from **grazed dairy** is increasingly sought by consumers because of perceived animal welfare advantages, grazing systems provide the farmer and the animal with unique challenges. The cow may have to walk long distances and has to be able to harvest feed efficiently in a highly competitive environment because of the need for high levels of pasture utilisation. She must be: highly fertile, with a requirement for pregnancy within ~80 days post-calving; 'easy care', because of the need for the management of large herds with limited labour; able to walk long distances for milking; and robust to changes in feed supply and quality, so that short-term nutritional insults do not unduly influence her production and reproduction cycles. In the future, there will be greater emphasis on more difficult to measure traits that are important to the quality of life of the animal in each production system and to reduce the system's environmental footprint.

An interesting example of the benefits of selective breeding for health traits is seen in Romania in a research project on buffalo[298]. The **Romanian Buffalo** is one of the most important genetic resources for milk and meat production in many countries across the globe. In this respect, it is quite important to develop efficient traits associated with health and production. The objective of this study was to estimate genetic parameters for milk somatic cells count (SCC). The results indicated that environmental factors have a greater contribution to the phenotypic manifestation of SCC performance. Therefore, the number of somatic cells is a result of milk hygiene and not included in the genetic selection of buffaloes (i.e. resistance to mastitis).

Pigs

Improving udder quality traits in **sows** can aid survival, health and lifetime performance of piglets.

Genome editing technology enhances the toolbox of trait-selective breeding. Methods for genome editing have developed over the past decades, making the technology more efficient and specific. Technology to generate **edited pigs** is developing alongside genome editors to generate productive and affordable animals much faster. For two major pig diseases, it has been shown that resistant animals can be generated that are refractory to infection. Two major hurdles still to be faced prior to the implementation of this promising technology concern the ethics of such technology, consumer acceptance and the regulatory framework it operates in[346].

A Spanish study[38] investigated the variation in Average Daily Gain (ADG) between pigs vaccinated with a local Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) strain and pigs infected with a wild-type virus. Pigs from negative PRRSV farms were infected with a wild-type virus or vaccinated with a local PRRSV strain. The amount of virus shed from the pigs, ADG and their genotype (i.e. 'WUR' at a specific protein gene) was assessed. Results showed individual variation in the amount of virus from pigs challenged with a wild-type or vaccine strain. The presence of the gene trait, WUR, was linked to positive ADG in vaccinated pigs. However, the reverse happened in a virus-free environment where pigs without this gene trait were those that grew fastest. There is scope for selecting pigs according to their responses to PRRS virus infection - the WUR gene trait may play a role in PRRSV resistance.

Poultry

Keyhole limpet hemocyanin (KLH)-binding natural antibody (NAb) titres in **chickens** are heritable, and higher levels have previously been associated with a better survival rate. This research suggests that selective breeding for higher NAb levels might increase survival by means of improved general disease resistance. Chickens were divergently selected and bred for total NAb levels binding KLH at 16 weeks of age for six generations, resulting in **a High NAb selection line and a Low NAb selection line**. To test for differences in disease resistance, chickens were challenged with *avian pathogenic Escherichia coli* (APEC) in two separate experiments. Overall, 50–60% reduced mortality was observed in the High line compared to the Low line for all APEC doses. In addition, morbidity was determined of the surviving chickens at 15 days of age. The High line had lower morbidity scores compared to the Low line. The authors concluded that selective breeding for high KLH-binding NAb levels at 16 weeks of age increases APEC resistance in early life. This study and previous studies support the hypothesis that KLH-binding NAb might be used as an indicator trait to selectively breed for general disease resistance in an antigen non-specific fashion. (Berghof, 2019).

Also, technology to generate **edited chickens** is developing alongside genome editors to generate productive and affordable animals much faster. In chickens there are promising laboratory results but no genome-edited, resistant chickens yet. Genome editing allows us to overcome bottlenecks in trait-selective breeding and allows the incorporation of genetic traits from other breeds, related species, or laboratory results[346].

How can breeding for resilience help reduce the need for ABs?

Breeding innovations that select for and multiply genetic traits that result in resilience to disease and environmental stressors, lead to healthier animals that are less likely to need ABs. From simple selective breeding based on animal performance or phenotypes so that only healthy animals pass on genetic material to their offspring, to molecular techniques, genome sequencing and gene editing in order to predict, choose and produce resilient livestock – breeding innovation can help reduce AB reliance in livestock production. Nevertheless, many of the newer technologies are fraught with ethical issues and require strict oversight to limit negative impacts.

5.8. Specific alternatives

Highlights

- Research on alternatives to antibiotics (ABs) has intensified greatly in the last two decades.
- In practice, there is still not a widespread use of alternatives within farms.
- However, there is an increased awareness by the farmers over the need to use prevention tools to help reducing the use of antimicrobials.
- In research trials, there seems to be a positive association with growth performance.
- Inconsistent results have been found in terms of therapeutic action. Specific approval processes and strict standardization methods are needed.
- The research to alternatives to ABs will be a long process.
- None of these alternatives are likely to fully replace AB use in animals in the foreseeable future but their use will be needed to establish a prudent use of AB.

What are specific alternatives to antibiotics?

During the last two decades, there is an increasing concern for the public health consequences of the sustained, long and increased use of ABs in livestock production. In response, an intensive amount of research has been focussing on the development of alternatives to ABs. The most widely researched alternatives include probiotics, prebiotics, acidifiers, plant extracts, bacteriophages and nutraceuticals such as copper and zinc (which meanwhile is being phased out). Less traditional alternatives have also been studied including host defence peptides, clay minerals, egg yolk antibodies, essential oils, eucalyptus oil-medium chain fatty acids, rare earth elements and recombinant enzymes.

In this chapter we will reflect on the main categories of alternatives that have been successfully used and present the latest applications of specific alternatives to farm animals that were found in the database of DISARM.

Why are specific alternatives relevant to reducing antibiotics and the DISARM network?

The use of specific alternatives to antimicrobial treatment has a straightforward link to reducing ABs as they were specifically introduced to the market to replace the latter. Moreover, the promotion of the use of alternatives to ABs is of utmost importance to the DISARM network as it serves the mission of highlighting preventive measures to combat disease and is expected to help farmers minimize AB use.

What do we want from alternatives to AB use (qualities, feasibility), and what are we concerned with?

The mechanisms of action and applications of various alternatives to ABs such as immunity modulating agents, bacteriophages (and their lysins), egg yolk antibodies, host defence peptides, pro-, pre-, and symbiotics, plant extracts, inhibitors targeting pathogenicity and feeding enzymes have been discussed in a number of reviews that deal with **pigs**[382, 391, 477, 478], **poultry**[23, 133, 388], **cattle**[187, 369] or **multiple species** of farm animals[28, 283, 284, 389, 479]. These reviews are highly recommended for further reading. Overall, there are certain properties that each alternative should minimally have (e.g. no side effects on the animal's health, be safe for the animal as well as for farm workers and public health, and be stable in the feed and/or the gastrointestinal tract). To standardise production, selection processes have been published to define the required properties of several alternatives (e.g. probiotics, prebiotics)[28]. Other qualities that an ideal alternative to ABs should have include: (i) be easy to eliminate from the body or result in residues only for the short-term, (ii) not induce bacterial resistance, (iii) be easily decomposed and not affect the environment, (iv) not negatively affect palatability, (v) not destroy the normal intestinal flora, (vi) kill or inhibit the growth of pathogenic bacteria, (vii) enhance resistance to the disease, (viii) improve feed efficiency and promote animal growth, (ix) have good compatibility and x) be economically feasible. In fact, there are no alternatives to ABs that currently meet all these requirements[283].

Moreover, the feasibility of these alternatives has been analysed as well. Several alternatives have shown promising results especially in terms of growth promotion[382]. In addition, optimization techniques (e.g. microencapsulation of essential oils) have helped to increase the bioavailability of active compounds[479]. Yet, several compounds produce inconsistent results in terms of therapeutic action and do no equal ABs in their effectiveness[283, 382]. The lack of clear regulations when developing and administering such products only adds to this inconsistency[52]. Consequently we need to focus a bit more on the concerns that were identified in the aforementioned reviews.

Concerns

Natural plant products are a particular case among the alternatives reviewed. Due to the diversity of bioactive components in these natural, non-purified preparations, their effective doses are difficult to determine and the effects on animals are not totally controlled[187]. Farmers and consumers alike generally perceive ‘natural plant extracts’ to be less toxic than ABs or other chemical products. However, this perception is scientifically questionable as there are many examples of dangerous natural toxins. The use of plant extracts as feed additives must therefore meet the same general requirements as non-natural products, e.g. they must be safe for both the animal and the handler of the product, they must not persist as residues in animal products, and they must not present a risk to the environment.

Though vaccines against viral diseases can help reduce the need for AB use by controlling the spread of secondary bacterial infections, several vaccines are available targeting specific bacteria. The OIE 2015 report concluded that new or more effective vaccines against the following pathogens would have a high impact on reducing AB use in the **pig** industry: *Streptococcus, Haemophilus, Pasteurella, M. hyopneumoniae, Actinobacillus, E. coli, Lawsonia, Brachyspira*, and bacterial infections secondary to PRRS, influenza, and Rotavirus. Also for **chickens** the list from the OIE 2015 report included *E. coli, C. perfringens,* coccidiosis and secondary bacterial infections related to viral infectious bursal disease or infectious bronchitis. New developments in selecting and potentially tailoring bacteriophages provide a promising avenue for controlling pathogenic bacteria without the need for traditional small-molecule ABs[387]. Whilst the efficacy of phage therapy varies according to the bacterial target and the complexity and location of the infection site(s), most recent studies in intensively-reared livestock have found that these pathogens can be significantly reduced using phages[387,388]. Pathogens which are more genetically homogeneous, such as *Staphylococcus aureus*, may be more attractive targets for phage therapy than genetically diverse hosts such as *E. coli*[388]. Phage therapy has been used to control Salmonella in **chickens** with varying degrees of success[388].

Thus, a gap in effectiveness and standardisation of processes between AB alternatives and current ABs is still evident[283]. Immunomodulators and feed enzymes mainly preserve the health of animals, but do not directly kill or inhibit bacteria. Bacteriophages have currently only a limited use in practice (two products approved by US-FDA), and there are numerous concerns over their safety as there is no official approval process to date neither by the FDA nor the EMA. The composition of plant extracts and probiotics is complex and their stability is limited, resulting in varying effects and safety risks. Inhibitors targeting pathogenicity of bacteria are still being studied with no approved products currently available, and most of these molecules being toxic to eukaryotic cells. Biofilm inhibitors show good results only when used in combination with ABs. Although host defence peptides can be used to treat bacterial infections, their high costs and narrow antibacterial spectrum restrict their wide use, and they can still induce bacterial resistance. Meanwhile, proteinaceous compounds, for example, feed enzymes that have been put into the market as well as bacteriophage lysins, enzymes and enzymatic biofilm inhibitors, are naturally unstable and easily degraded in the digestive tract.

When reviewing the literature, the general conclusion is that none of these alternatives are likely to fully replace AB use in animals in the foreseeable future[283]. Therefore, prudent use of ABs and the establishment of scientific monitoring systems are the best and fastest way to limit the adverse effects of AB overuse.

Applications of specific alternatives in research and practise

Pigs

To assess the effectiveness of alternatives to reduce AB use, 68 **farrow-to-finish pig** herds located in Belgium, France, Germany and Sweden were recruited on a voluntary basis to implement tailor-made intervention plans to reduce antimicrobial use (AMU) focusing on biosecurity, feed management and welfare[135]. When implemented, a substantial reduction of AMU in pig production was achievable without jeopardizing animal health. The AMU reduction in the youngest age categories (suckling and weaned pigs) and the reduction of group treatments via feed and water was in line with recent European Guidelines on the prudent AMU in veterinary medicine.

A Korean study focused on the effects of eucalyptus-medium chain fatty acids (E-MCFAs), zinc oxide (ZnO) (which is currently being phased out), and AB on performance, nutrient digestibility and serum chemistry parameters of **nursery pigs**[287]. Three experiments were conducted. Weaned pigs were given five treatments consisting of a basal diet or the basal diet supplemented with AB (33 mg per kg tiamulin and 44 mg per kg lincomycin), ZnO (1500 or 2500 mg per kg), or 0.1% E-MCFAs (Experiments One and Two). In Experiment Three, 1% diatomaceous earth was added and the negative control was not used. In all three experiments, performance of pigs fed the four supplemented diets did not differ. Digestibility was higher in the diet supplemented with E-MCFAs than in diets supplemented with ZnO or ABs. This study indicates eucalyptus-MCFAs can be successfully used as a growth promoter in diets fed to nursery pigs.

Poultry

Poultry face several challenges potentially disturbing normal functioning. Especially for **broilers**, the gastrointestinal tract may be affected by the intensive production rates resulting in weakened absorption of nutrients, reduced performance and increased mortality. Therefore, in the past ABs were supplemented to the birds diet to make them cope better with harsh conditions. With the ban on ABs as in-feed growth promoters farmers lost an effective tool to help birds achieve their performance capacity. Phyto biotics seem to be the most promising alternatives to ABs as growth promoters as they are of a natural origin and generally regarded as safe, although, they contain a broad number of pharmacologically active substances with unknown action[392]. Experiments with various Phyto biotic products conducted with **broilers** have shown a tendency for improved performance and health status, although the net margin amounts only to a few percent compared to control groups[133].

The importance of developing alternatives to AB use was reflected in a scientific review paper presenting an overview of publications on alternatives to ABs for **poultry** production over the course of only one year (2016)[503], finding 134 relevant publications over the observation period (1 year). In total, 18 categories of products were identified, with a dominance of publications on probiotics and plant extracts. The most common aims were to show a zootechnical effect, show a sanitary effect, combat coccidiosis/necrotic enteritis and/or achieve gut colonization. In total, 20-25% of the published trials failed to show any effect towards the selected markers.

The objective of a French study was to develop and test a simple, reliable and repeatable method to determine capacities of plant extracts in stimulating the natural defences of **poultry**[258]. A bibliographic study identified 4 plant extracts: Astragalus, Echinacea, Ginseng and Melis*sa*. A metabolic activity test was carried out on three **chicken** cell lines, representative of the respiratory (CLEC213), hepatic (LMH) and immune (HD11 macrophages) functions. Immuno-stimulation was studied in macrophages by assessing the production of the pro-oxidant and antimicrobial molecule nitric oxide and the activation of the pro-inflammatory NFκB pathway. Astragalus, Echinacea, Ginseng and Melissa had little or no cytotoxic effects. Results from experiments with Melissa and Ginseng suggest these may have immune stimulating activities. The Melissa and Ginseng extracts appear to be non-cytotoxic candidates, capable of stimulating the natural immunity in poultry.

Several essays to test alternative practices to reduce bacterial contamination of freshly laid **eggs** were performed by a French research group[270]. During cooling of freshly laid **eggs**, the porosity of the eggshell allows environmental microorganisms to penetrate the egg resulting in poor chick health. The presence of microorganisms on the shell, the time between laying and collection, and the storage conditions of eggs can increase the risk of contamination. The disinfection of hatching eggs is an effective prevention method, but it is done several hours after egg laying, with some risk of bacterial proliferation. Therefore, it is important to reduce the contamination of the eggs after laying and before their introduction in the hatching process. Using air disinfection systems in the egg conveyor didn't lead to a decrease in bacterial contamination of the egg surface. However, quick isolation of freshly laid eggs from the rearing area led to a significant decrease of outer- and inner-shell bacterial contamination.

A *Lactobacillus plantarum* strain was isolated from **chicken** faeces and assessed for its probiotic use in Algeria[4]. The administration of *L. plantarum* S27 to each chick daily by gavage, for 31 days, improved food intake and weight gain. Supplementation of *L. plantarum* S27 resulted in increased live body weight as of the 4th week, compared to chickens from group 1 (preventive AB treatment with erythromycin) and group 2 (control group without treatment). Remarkably, weights of carcasses, heart and gizzard from the probiotic group were significantly higher. *In vitro* and *in vivo* analyses indicated that *L. plantarum* S27 is a potentially effective probiotic for chickens to reduce AB use in animal feed. This study underlines the potential of using the chicken's digestive tract as potential source for probiotic strains.

Another French study aimed at assessing the efficiency of several alternatives to ABs on technical performances and health status of **Label Rouge broilers**, produced in sub-optimal conditions (egg storage before incubation for 18 days and a less digestible diet with a deficiency in essential amino-acids)[267]. During the growth period (i.e. 15-76 days of age), four preventive treatments were tested: essential oil, symbiosis between prebiotic and probiotic, clay-algae association and finally an organic-acid-polyphenol complex. The (day-old) chick quality, measured according to eight criteria, was negatively impacted by storage duration before incubation. The supplementation with the various alternatives to ABs did not result in a statistically significant difference regardless of the prophylactic treatments.

Cattle

A field lab aiming to improve the health of **dairy cows** through a reduction in AB use, particularly for the treatment of mastitis was organized by the Organic Research Centre "Elm Farm" in the UK[3]. Participants trialled the use of Uddermint®, a liniment cream containing 35% mint oil, to reveal its efficacy in reducing high somatic cell counts (SCC), an indicator of subclinical mastitis. Six farmers participated in the experiment. Newly-calved **cows** were treated with Uddermint® and the results for both treated and untreated cows were reported. SCCs were checked using National Milk Records (NMR). Although there was large variation in results between treatments and participating farms, when farm data was combined, SSCs were significantly lower in the cows treated with Uddermint®. As a result, Uddermint® may be used as a complementary treatment for mastitis in less severe cases, perhaps able to reduce AB usage.

The search for alternative drugs based on plants has become a priority in livestock medicine. In this context, the main objective of a Romanian study was to determine the antimicrobial effect of plant extracts and products on pathogens isolated from **bovine** mastitis[280]. A total of eleven alcohol extracts and eight plant-derived products were tested using 32 microorganisms found in milk. The results showed an inhibition of bacterial growth for all tested plants, with better results for *Evernia prunastri, Artemisia absinthium*, and *Lavandula angustifolia*. Moreover, *E. prunastri, Populus nigra*, and *L. angustifolia* presented small averages of minimum inhibitory and bactericidal concentrations. Among the plant-derived products, three out of eight showed a strong anti-microbial effect comparable with the application of florfenicol and enrofloxacin. The results of this *in vitro* study suggest an important anti-microbial effect of these products on pathogens isolated from bovine mastitis with a possible applicability in this disease.

A US study investigated the effect of 2 alternative therapies, lactoferrin (an iron-binding protein found in colostrum) and cinnamaldehyde (an essential oil of the cinnamon plant) on growth, disease incidence and mortality in **veal calves** (1 g/d in milk replacer)[124]. Body weight and average daily gain were similar across treatments. Neither lactoferrin nor cinnamaldehyde had an effect on diarrhoea incidence. However, the risk of navel inflammation was significantly lower for calves that received cinnamaldehyde compared with calves in the control group. Additional research is needed to confirm the effect found and compare various doses of these alternative therapies on calf health and growth, in addition to different routes of administration.

A US study was set up to evaluate the efficacy of two non-AB treatment options for digital dermatitis in **cows**. A topical application of copper sulphate and iodine (CUI) was compared to the topical of honey and iodine (HOI) or a control group (CON)[340]. A randomized clinical trial was conducted using 70 multiparous Holstein cows with an early digital dermatitis lesion at a certified organic dairy farm in Northern Colorado, USA. The two non-AB formulations resulted in an earlier transition to mature lesions compared with the control group. The CUI combination was the most effective treatment in reducing lesion size, pain and lameness in affected cows. However, this combination had short-term efficacy, which did not persist throughout the duration of the study. The HOI combination produced only transient reduction in lesion size.

A French study evaluated an essential oil mixture for the treatment of mild and moderate clinical mastitis in **dairy** cattle[498]. In dairy herds from Brittany, Pays de la Loire or Auvergne-Rhône-Alpes, a total of 131 clinical cases were randomly assigned to essential oil or AB group. An essential oil mixture was applied on infected quarters during 14 consecutive milking rounds. Results showed that clinical cure rate was lower in the essential oil group than in the AB group (72.3 % vs 88.1 %). Cure rate based on two consecutive individual SCC measurements (< 300 000 cell/ml after occurrence) was higher in the essential oil group only in Brittany, Pays de la Loire (95.0 % vs 60.0 %). Bacteriological cure rate was lower in the essential oil group compare to the AB group only in Auvergne-Rhône-Alpes (96.0 % vs 53.4 %). In the growing context of antimicrobial resistance, results showed that there is an interest to consider essential oils as a complement to AB to evaluate new treatment strategies of mammary infections in dairy cattle.

The udder cleft incidence on a farm is often underestimated, particularly on farms with a milking robot. Udder cleft issues in **cows** like udder cleft dermatitis or foul udder are often located between the front teats and at the transition of the front quarters and the abdominal wall. Although this condition is well known in the **dairy** industry, not much research has been performed on the subject. In the Netherlands, a large-scale study demonstrated the positive effect of a non-AB product (Intra Repiderma© spray [Intracare BV, The Netherlands]) on mild udder skin issues[401]. Spraying of the mild cases every 2 days resulted in 81.8% full skin recovery with a median time of 4 weeks to recover.

A Danish research team studied the effect of probiotic “Zoolac® Bovimix Milk” fed to **calves** from birth until 4 weeks of age[415]. The product contained live yeast (*Saccharomyces cerevisiae*) and a postbiotic (i.e. immunomodulatory molecule produced by bacterial fermentation) consisting of *Lactobacillus acidophilus*. There was no effect on the health of the calves since both the control group and the experimental group had similar frequency of AB treatments. However, the calves supplemented with the product containing probiotic and postbiotic had a significantly better growth performance.

A national survey in France was conducted among specialized breeders, technicians and veterinarians to identify the perceptions, fears and motivations regarding the use of complementary and alternative medicines[495]. More than half of the breeders declared that they are already using alternatives to AB on their farms, mainly for the care of digestive and respiratory disorders. The main reasons for using these alternatives are on the one hand a desire to reduce AB use and on the other hand personal convictions. However, the lack of information and support is hampering professionals. Solutions must be found in the training of breeders, technicians and veterinarians who frequently express such a need.

Other

An *in vitro* Romanian study tested the antimicrobial action of the propolis tincture on six bacterial strains[200], namely three collection strains (*S. aureus* ATCC 25923*, Streptococcus pyogenes* ATCC 19615*, Salmonella typhimurium* ATCC 14028) and three strains isolated by the researchers (*S. aureus, Pseudomonas aeruginosa* and *E. coli*). Raw propolis tincture inhibited the growth of bacterial cultures in four of the strains. This study demonstrated that the propolis tincture has some antibacterial properties *in vitro*.

A Chinese study showed the protective effect of a beetle (*Zophobas morio)* hemolymph on **bovine** mammary epithelial cells[370]. The *Z. morio* hemolymph directly killed both Gram-positive and Gram-negative bacteria through membrane permeation, and prevented the adhesion of *E. coli* or the clinically isolated *Staphylococcus simulans* strain to bovine mammary epithelial (MAC-T) cells. In addition, *Z. morio* hemolymph downregulated the expression of nucleotide-binding oligomerization domain (NOD)-like receptor family member pyrin domain-containing protein 3 (NLRP3), caspase-1, and NLRP6, as well as inhibited the secretion of interleukin-1 beta (IL-1 beta) and IL-18, which attenuates *E. coli* or *S. simulans*-induced pyroptosis. Overall, the results indicated the potential role of *Z. morio* hemolymph as a novel therapeutic candidate for bovine mastitis.

Many farmers are engaged in small experiments about animal health. This paper offers a first characterization of experiments by 40 **dairy**, **pig** and **poultry** farmers working in **organic, Quality labelled systems or conventional systems**. We found that farmers carry out multiple *in situ* tests with alternative medicines. There is a clear tendency of transferring positive results for a given disease complex to one another. Seven portraits of farmers are presented to shed light on complementary dimensions of experiments: the appeal of novelty, the role of vets and technicians, and the role of farmer groups and training. It can be concluded that much can be learned from ethnographic investigations in order to grasp what farmers are experiencing when they endeavour to solve animal health problems.

5.9. Antimicrobial use reduction strategies

Highlights

- General enhancement of animal health and welfare can reduce the need for antibiotic (AB) use through better biosecurity, management and husbandry, facility design and management, and training of personnel, veterinarians and advisors.
- Specific alternatives to ABs include vaccination, feeding approaches and breeding.
- Changing attitudes, habits and human behaviour (farmers, agri-advisors and veterinarians) and improving information dissemination can contribute to reduced AB use.
- Benchmarking progress optimises success.
- Plan, do, check and act!

What are antimicrobial Use (AMU) reduction strategies?

AMU reduction strategies are initiatives, projects and programmes to reduce the need for and use of antimicrobials (particularly ABs) in livestock farming, in response to the global challenge of antimicrobial resistance (AMR). AMR development and spread is driven by human behaviour, from the prescription of antimicrobials to infection prevention and control. Resistant bacteria can be introduced and spread in the environment in many ways, such as the application of livestock manure as fertilizer. This complexity necessitates highly interdisciplinary research and policy making, comprising stakeholders from human health, animal health, and wider environmental health; this is called the One Health approach.

Antimicrobials play a crucial role in animal health, animal welfare and food-safety. However, a yet to be defined share of the burden of AMR is attributable to the use of antimicrobials in livestock production and there have been widespread efforts to address overuse and misuse. Nevertheless, responsible AMU should be and is practiced widely across European livestock production, hence, the ultimate goal is not necessarily to move towards zero use in all cases and all farms, but to stimulate prudent use (which does imply a reduction in total use at country and sector level). More on the prudent use of these essential medicines can be found in the next chapter on prudent use.

Our understanding of AMR in livestock production is hampered by the lack of AMU monitoring and surveillance data in many countries. In line with the WHO global action plan on AMR, research should be prioritized toward understanding the social/behavioural drivers of AMU, risk factors for AMR, establishing/improving systems to monitor AMU and AMR, and encouraging a holistic approach to AMR through the One-Health concept[303].

Why are AMU reduction strategies relevant to the DISARM network?

DISARM is a thematic network on disseminating innovative solutions for antimicrobial resistance (AMR) management. Whereas innovative solutions are often thought of as single (technical) innovations, e.g. feed additives, alternative medicines and vaccines, it is important that these innovations are disseminated and used in a coherent and holistic strategy at national and sectoral level. Such a coherent approach, involving public and private actors and stakeholders, can stimulate the use of innovative solutions and can help in deciding on the best combination of innovative solutions, adapted to each regional context, sectoral specificities, and ultimately even to farm-specific conditions.

International organizations, such as the World Organisation for Animal Health (OIE), World Health Organization (WHO) and the European Medicine Agency (EMA) have developed guidelines to help national authorities to set up their own strategies and measures to reduce the occurrence and spread of AMR[208]. These national measures and initiatives will act as a framework for those prescribing and using ABs in livestock production. Knowing the drivers of AMR, as well as reviewing when and where ABs are used, can be a useful starting point for veterinary practitioners and farmers alike.

While antimicrobials have been regular supplements in animal feed to maintain health and improve productivity of livestock, their overuse in feed has contributed to a rise in AMR globally. A review from China summarizes the current use of antimicrobials in livestock, the harmful effects of AMR, and comprehensive measures to combat misuse and overuse[442].

An annual review titled '*Industrial food animal production, antimicrobial resistance, and human health*'[439] calls for radical change in AMU in livestock production, controversially demanding complete cessation of use. The report highlights the following high-risk areas where action is needed:

1. The use of antimicrobials as feed additives;
2. Exposure of farmers, farm workers, rural communities and the general public to AMR pathogens, as well as contamination of air, water and soils near food animal production sites;
3. AMU in food production risks expanding the reservoirs of resistance because these genes can be transferred widely among microbial communities;
4. Reducing or banning agricultural AMU can reduce risks of AMR in the food supply chain;
5. Disposal of animal waste as a major route of environmental contamination by antimicrobials and resistant bacteria;
6. Farmers and farm workers are at significantly increased risks of infection by antimicrobial-resistant bacteria; they may serve as entry points for the general community and transfers into health care settings.

The following general measures can be taken to prevent emergence and spread of AMR relevant to livestock production: reduce and rationalise all AB use, implement infection control measures at farm level, develop strategies to mitigate the risks of antimicrobial residues and AMR bacteria in the environment, utilise rapid tests for diagnosis of infections, promote research on prevention and surveillance of AMR, develop novel antimicrobial agents, find alternative solutions for persistent health problems and improve public awareness of responsible AB use and risks associated with increased AMR[129].

When and where have AMU reduction strategies been implemented and by whom?

Concern about AMR did not develop evenly across the world but instead gave rise to an international patchwork of different regulatory approaches. *'Pharming animals: a global history of ABs in food production (1935–2017)'* traces the origins, global proliferation, and international regulation of agricultural AMU[448]. It argues that policymakers need to remember the long history of regulatory failures that has resulted in current AB use. For effective international stewardship to develop, it is necessary to address the economic dependencies, deep-rooted notions of development, and fragmented cultural understandings of risk, which all contribute to drive global AB consumption and AMR.

In 2017, EFSA and EMA jointly reviewed measures taken in the EU to reduce the need for and use of antimicrobials in food-producing animals, and the resultant impacts on AMR. Some reduction strategies have been implemented successfully in some Member States, including national reduction targets, benchmarking of AMU, controls on prescribing by veterinarians and restrictions on use of specific critically important antimicrobials, together with improvements in animal husbandry, disease prevention and control measures[131].

Advice on monitoring and using ABs as well as legislation, current situation and recommendations for the future are discussed on the following European-wide website (in Dutch and French, https://www.amcra.be/nl/adviezen-en-wetgeving), with a PDF per topic and can be found on the DISARM website[42].

At the European Commission (EC) level, legislation has been issued for the monitoring of AMR and these rules are applicable to each Member State with audits carried out where countries have developed programs on AMR that go beyond the legislation[209]. A study analysed existing data reports, legislation and recommendations on AMR and found that a “good practice guide” can be achieved by Member States with extensive experience in this area, which can be used to harmonize AMR programs within the EU.

In the UK, pharmaceutical companies have reported the quantity of authorised veterinary ABs sold throughout the country to the VMD (Veterinary Medicines Directorate; Governmental body) since 1993; this has been a statutory requirement since 2005[181]. Sales of veterinary ABs for use in food-producing animals for the year 2018, adjusted for animal population, were 29.5 mg/kg; a 3 mg/kg (9%) and 33mg/kg (53%) decrease since 2017 and 2014, respectively.

EPRUMA (European multi-stakeholder platform that facilitates and promotes a co-ordinated and integrated approach on the Responsible Use of Medicines in Animals) best practice guidelines for AB use combines a holistic and specific approach to maintain and improve animal health at specific sector level and at individual farm level[164]. The main objective of this combined approach is to balance the different elements that may have an impact on animal health, e.g. nutrition, housing, production, etc. Tailoring to the local situation of an individual farm and enabling final implementation by the farm owner/animal caretaker and other professional visitors to the farm (e.g. veterinarians, feed and husbandry experts) is key. The document also includes a decision tree for the responsible use of veterinary ABs.

A widely acclaimed UK report presents the key steps to reduce AB use in agriculture and the environment[100]:

1. Agree on targets to reduce AB use in food production to an acceptable level per kilogram of livestock and fish, together with limitations of use of ABs that are important for humans;

2. Agree on minimum limits for AB environmental waste, which should be taken into consideration by pharmaceutical companies, healthcare buyers and regulatory agencies everywhere;
3. Improved surveillance at an international level to monitor AMU and waste, and evaluate progress towards global targets.

Interventions in agriculture that could reduce AMU include improvements in infection control, better animal husbandry practices (e.g. less overcrowding, less stress, better welfare), greater/optimized use of vaccines and the adoption of diagnostic devices to improve veterinary prescribing practices.

The implementation of disease control programs on farms requires changes in behaviour. A study involving 43 interviews with farmers in England and Wales explored the perception of responsibility for zoonotic disease control among cattle farmers and identified barriers to implementation of control programs[318]. Younger farmers and/or larger herds were more likely to place financial responsibility upon the industry rather than government. Farmers with no intent to adopt control measures identified their private veterinarian as the preferred motivator, whereas consumer-demand and financial rewards or penalties were significantly associated with farmers who intended to control AMU.

The Responsible Use of Medicines in Agriculture Alliance (RUMA) in the UK is an alliance of over 26 industry organisations representing every stage of food production from "farm to fork". RUMA aims to promote a co-ordinated and integrated approach to best practice in the use of medicines for farm animals[116]. Responsible use of ABs on farms means using ABs as little as possible and as much as necessary. Regardless of the farming system, the focus for improved animal husbandry should include improved biosecurity practices and on-going veterinarian and farmer training on disease prevention and the responsible use of ABs.

The discovery of significant reservoirs of AMR pathogens in the Netherlands led to a successful collaboration between the government and stakeholders to reduce AMU in farm animals[41]. Total AMU in farm animals in the Netherlands decreased by 56% in the period 2007–2012. A combination of compulsory and voluntary measures, and reduction goals resulted in this decrease.

Since 2011, the consumption of veterinary AMU has been recorded in the Netherlands[64]. These data are used to define benchmark indicators and presents the results of sector-wide consumption of antimicrobials, for all **pig**, **veal calf**, and **broiler** farms. The distribution of antimicrobial consumption per farm varied greatly within and between farm categories. The insights obtained from the this study, and the full transparency obtained by monitoring AMU per farm, has helped reduce AMU and implement antimicrobial stewardship.

France has also reduced AB use in veterinary medicine starting with a 25% reduction in ABs between 2010 and 2014[14]. Topics such as critically important ABs (3rd or 4th generation cephalosporins and Fluoroquinolones), age at treatment and disease diagnosis have all been focal areas. The French '*Ecoantibio plan'* has enabled a reduction of 39% in veterinary ABs in 6 years, all animal sectors combined[34]. The 1st Ecoantibio plan 2012-2016 aimed to reduce the use of ABs in animals by 25% in 5 years. The 2nd Ecoantibio plan 2017-2021 aims to consolidate this result by continuing efforts. Two million euros per year are devoted to research projects, training and awareness campaigns. The implementation of the Ecoantibio plan has led to a strong mobilization of the various partners in **cattle** breeding and the agricultural profession in the cattle industry[188].

An article from Romania entitled '*The importance of databases to manage the phenomenon of resistance to antimicrobials for veterinary use*'[99] details the key organisms involved in AMR, and principal reference data for AB consumption across livestock and humans. Databases and surveillance systems like these contain more and more data and are enabling researchers to fight AMR.

Looking outside Europe, the Canadian government has developed a One Health strategy to address AMR across the country[118]. Veterinarians are key to antimicrobial stewardship and are advised to use the 5 Rs in their daily prescribing duties:

1. Responsibility
2. Reduction
3. Refinement
4. Replacement
5. Review

There have been several policy changes in Canada in recent years (2018-2019) pertaining to the prescription and purchasing of antimicrobials. Animal owners will no longer be able to import medication for use on their own animals and they will need to purchase antimicrobials from a veterinarian, pharmacy or feed mill within Canada. Additionally, a valid VCPR (Vet-Client-Patient-Relationship) must be established prior to a veterinarian providing services to clients or their animals. Health Canada is also increasing its oversight of medically important antimicrobial Active Pharmaceutical Ingredients (APIs) for veterinary use, including their import, manufacture and distribution.

The *Canadian Council of Chief Veterinary Officers' (CCVO) Antimicrobial Use in Animal Agriculture Committee* established an AMU Surveillance Working group in October 2013[138]. The overarching objectives of this group were to (1) review current Canadian non-human AMU surveillance programs, (2) compare these programs to AMU surveillance programs in other countries, and (3) formulate recommendations and options for non-human AMU surveillance in Canada.

A 2019 update reflects on the Canadian One Health strategy based around the pillars of Surveillance, Infection prevention and control, Stewardship and Research & Innovation, and evaluates what progress has been made regarding the recommendations[121].

Monitoring and surveillance of AMR

In a review from Spain, important examples of AB resistance in microbes of concern for human health are described, and the process that led to their development is presented[241]. The report begins by describing the resistance genes, the genetic elements involved in the maintenance and dissemination of AMR, and ends with other critical factors that contribute to its spread. Possible responses to the problem are reviewed, with special reference to the development of new ABs.

A Romanian study found 36 *Salmonella* serotypes in 1357 *Salmonella*-positive samples in 2015, and 28 serotypes in 1188 samples in 2016. More details about Salmonella surveillance in Romania can be found in the study but in order to reduce zoonotic serotypes, continued collaboration is needed of all professionals involved in food security[205].

Evidence indicates that AB use history and co-selection of resistance are key elements for perpetuation of resistance[27]. Data suggest that recent Dutch policies aimed at reducing total AB use and restricting the use of critically important AB have decreased *E. coli* resistance in the **pig** and **veal calf** production sectors while the impact on AMR in the **dairy** cattle and **poultry** sectors is an emerging picture. More recent research from the Netherlands estimated the herd-level prevalence of AMR bacteria, specifically *ESBL- and AmpC-producing E. coli* among Dutch **dairy** farms[53]. No association was found between the total AMU and the ESBL/AmpC herd status. The use of third- and fourth generation cephalosporins, however, was associated with an increased odds of having a positive ESBL/AmpC herd status and seems important in reducing ESBL/AmpC mediated resistance. Four other management factors were also found to be associated with the ESBL/AmpC status of dairy herds: treatment of all cases of clinical mastitis with antimicrobials, a higher proportion of calves treated with antimicrobials, not applying teat sealants in all cows at dry off, and the use of a floor scraper. Also, *ESBL/AmpC E.coli* could frequently be cultured from slurry samples collected from Dutch dairy farms.

Between 2011 and 2013, the period during which the use of 3rd- and 4th-generation cephalosporins was minimized in the Netherlands, the between-herd prevalence of resistant *E. coli* expressing ESBL/AmpC-genes in Dutch dairy herds declined significantly[67]. **Calves** were found to have both a much higher individual animal prevalence and a higher level of shedding than young stock and cows. The most sensitive approach to find *ESBL/AmpC positive E. coli* in Dutch dairy herds is through collecting samples from individual young calves.

Genes transferred by plasmids are important vehicles for the spread of AB resistance in two groups of bacteria, *Enterobacteriaceae* and *Pasteurella*[233]. The identification of plasmid characteristics and their association with humans and animals provides important information - it is essential to understand the contribution of these plasmid-borne genes to the transmission of resistance. An international review provides an overview of all known AMR-related types of genetic elements (e.g. plasmids) in *Enterobacteriaceae*, the resistance genes they carry and their geographical distribution[235]. Understanding how these genetic elements function and spread can lead to better understanding of how AMR is being spread.

Research shows that the ABs most often found in livestock manure are tetracyclines, sulphonamides, macrolides, quinolones and fluoroquinolones[107]. These can later end up in the environment. Animals discharge 30–90% of administered ABs unchanged or as active metabolites. During storage, most ABs form complexes with soluble organic matter and remain fairly stable. After spreading, soil can have some protective effect. This paper reviews the ways in which ABs can be removed by enhanced slurry management, including administration of drugs to livestock and treatment of slurry/manure to remove ABs, especially composting.

Measuring AMU

To quantify AMU and measure progress against targets, various types of measures are available, all with advantages and disadvantages. These are referred to as indicators of AMU and are described in detail in a French paper that shows that end results can differ substantially depending on the method chosen[108].

The 8th ESVAC (European Surveillance of Veterinary Antimicrobial Consumption) report presents data on the sales of veterinary antimicrobials from 30 European countries in 2016[180]. The report describes changes in consumption of veterinary antimicrobials for the years 2010-2016 and focuses on the changes across time in each country.

Standardized statistics are necessary for international comparison of usage, monitoring of national drug usage and for comparative studies of drug use, as demonstrated by 'VetStat' in Denmark[79]. The defined animal daily dose (DDDvet) is a better measure than the weight of active compound, but interpretation of trends in drug consumption also should include current knowledge of changes in the prescribed daily doses. To enable direct comparison of usage, the number of animals in the target population should be used as a baseline. Depending on the scope, information on number of animals may be obtained from different sources.

A comprehensive report by DANMAP, the *Danish Integrated Antimicrobial Resistance Monitoring and Research Programme*[95] presents the results of monitoring AMU and AMR in food animals, food and humans from 2017. The report summarizes the result of susceptibility testing of isolates obtained by hospitals, general practice, veterinary practice and the National Food and Veterinary Authority, as well as records of types and number of antimicrobials prescribed by veterinarians.

The next section will give an overview of species-specific examples of AMU reduction strategies from across the globe.

Cattle - dairy and veal

An EU Horizon 2020 project report highlighted the extent to which total AMU and Critically Important AB (CIA) use can reduce across the European **dairy** sector. EuroDairy gathered intelligence through its partners and wider networks, organized a cross border workshop, farmer exchange visits, technical webinars, and linked to regional Operational Groups focussed on reducing ABs[371]. The best way to sustainably reduce the risk of AMR is through an integrated approach to disease control, with hotspots for AB use being mastitis (including dry cow therapy), lameness and foot health, respiratory disease and young stock health.

The International **Dairy** Federation (IDF) has produced 2 factsheets on responsible AMU. The first one describes what AMR is, outlines dairy sector guidance on prudent use of antimicrobials and defines the global dairy position on AMR[92]. Guidelines for dairy farmers include:

- Implement biosecurity measures to prevent introduction of diseases and resistant bacteria onto the farm;
- Implement a management programme for chronically infected cows;

- Implement an effective herd health management programme;

- Avoid feeding milk-containing residues to calves or other animals on the farm;

- Ensure that withdrawal times set for the antimicrobials are respected before the milk from treated animals is used for human consumption.

The second IDF guide describes prudent use of antimicrobial agents in **dairy** production[93]. It contains a chapter with definitions of used terminology, complemented with descriptions of 'Good practices' for dairy farmers, veterinarians, food processing companies, pharmaceutical companies and competent authorities. The focus of this comprehensive publication is on desired outcomes rather than on specific prescriptive actions or processes.

In the UK a bottom-up approach has been assessed for reducing AMU in **dairy** farming[310]. Five Farmer Action Groups were established and followed for 2 years to understand how a participatory approach helped to achieve practical, farmer-led changes to reduce AMU reliance on their farms. Medicine reviews, benchmarking and a co-created, practical action plan helped each farm to assess change in AMU. 70% of farms reduced highest priority critically important AMU over the 2 years. Knowledge gaps were identified by the farmers, particularly issues around knowledge mobilisation between veterinarians and farmers at the time of the study. The facilitators supported the knowledge mobilisation and helped build a sense of solidarity within the groups.

In order to identify factors associated with high AMU and set a threshold for AMU (benchmarks) for **dairy** cattle, data was gathered from 358 UK dairy farms using various methods[77]. Data analysis indicated that usage of ABs via oral and footbath routes increased the odds of a farm being part of the top AB users. While dairy cattle farm AMU was apparently lower than UK livestock average, some farms had extremely high AMU. Identification of these high use farms can be effective in targeting AMU reduction strategies and help reduce overall dairy cattle AMU.

To decrease AB usage sustainably, it is considered crucial to change farmer mindset[68]. Based on models from social psychology, the RESET Mindset Model was created in the Netherlands involving several different actions, both voluntary and compulsory. An independent veterinary medicine authority and a national database on AB usage was also developed (MediRund), which enabled transparency and benchmarking on AB usage. This was done together with other activities, such as herd health and treatment plans, selective dry cow therapy, and the strong limitation on the use of critically important ABs. As a result, AB usage at the herd level, referred to as the 'AB number', became an important and socially-accepted herd level parameter and AB usage in dairy cattle in the Netherlands decreased significantly.

Since 2015, veterinarians in Austria have been required by law to report antimicrobials dispensed to farmers for use in food-producing animals. The study presented here collected data on antimicrobials dispensed to farmers and those administered by veterinarians[80]. Results show that **dairy** cattle in the study population in Austria were treated with antimicrobial agents at a relatively low and infrequent defined daily dose rate. The most frequently used antimicrobial group with respect to mastitis treatments was the betalactams, primarily penicillins, with third and fourth generation cephalosporins being the most commonly used highest-priority critically-important antimicrobials.

A Danish study analysed database recordings of milk yield and somatic cell count (SCC) from routine milk recording schemes for 518 **dairy** herds in Denmark[315]. Analysis was performed to identify the main factors for treatment in different groups of farms. The results showed that the most important factors for predicting AB treatments vary from one farm to another. Health indicators such as test results or SCC were most indicative for treatment on some farms, whereas other groups seemed to depend more on production factors (milk yield) or later culling of the cows.

A study involving 18 Latvian farms and 180 samples aimed to detect the prevalence and AMR of *Escherichia coli, Enterococcus* spp., and other common pathogens [194]. Of these, 64% *E. coli*, 100% *Enterococcus faecalis* and 96% *Enterococcus faecium* isolates were resistant at least to one AB. The prevalence of *extended-spectrum β-lactamase (ESBL)/AmpC-positive E. coli* were 11.1%. Farm size, bought-in calves, contact with other calves, and antimicrobial treatment of cows were associated with increased prevalence of resistant *E. coli* and *Enterococcus* spp. Despite low AMU in Latvia, high rates of AMR in faecal indicators and *Campylobacter*, combined with high prevalence of ESBL-positive *E. coli*, show the necessity for prudent AMU.

Outside of Europe in Argentina, AMU in **dairy cows and calves** was estimated using standardized drug usage indicators[62]. In lactating dairy cows, intramammary usage accounted for the majority of total drug usage. All the surveyed disease cases in calf rearing units included treatment with ABs.

'CalfOK' is a Dutch initiative that provides insights into the rearing of all **calves** born on the dairy farm aiming to improve their health and welfare[89]. Since 2018, every dairy farmer can request a CalfOK score for the farm. CalfOK is composed of data on births and rearing, the use of ABs in calves and the herd health status. In total there are 12 key figures, and each farm can score a maximum of 100 points. The result provides insight into the quality of calf rearing at the individual dairy farm, and also makes a comparison with other farms.

National policy in the Netherlands from 2010 aimed to reduce AMU in food-producing animals[51]. In the context of **veal** production, a study evaluated 2 different strategies: 1) only reducing antimicrobials and 2) reducing antimicrobials with a cleaning and disinfection program. These results suggest that AMU reduction might be a good strategy for reducing *methicillin-resistant Staphylococcus aureus* (MRSA) in veal calf farming. However, the cleaning and disinfection protocol used in this study was not effective. The study indicates that the long-term AMU decrease is likely to lower MRSA levels in people living and/or working in veal farms.

Based on collaboration between researchers, farm management and consulting veterinarians, a new policy was implemented to reduce AMU in **calves** in the US[25]. The effects of policy changes on AMU and on AMR in commensal *Escherichia coli* was investigated. In general, there was a declining trend in resistance to most antimicrobials during and after policy changes were implemented, except for ampicillin, ciprofloxacin, ceftiofur and gentamicin.

A simple tool that can help farmers reduce AMU is a calculator for the approximate energy requirements for a pre-weaned **dairy heifer** and the amount of energy provided by milk replacer[178]. This article highlights the importance of sufficient feeding for preventing disease in calves and therefore the risk for increased AMU.

Many studies measure AMU on dairy farms, but little is known about the quantity and the way antimicrobials are stored on farms. To better understand this situation, data were collected from 27 **dairy** farms in England and Wales[83]. Antimicrobials were the group of medicines most commonly stored. It was common to find expired medicines and medicines not licensed for use in dairy cattle, and of antimicrobials considered critical.

The relationship between AMU and the occurrence of AMR in cattle was investigated in a longitudinal study in Belgium[112]. Three types of production system were investigated for 2 years, for a total of 25 herds: 10 **dairy**, 10 **beef**, and 5 **veal** herds. Two different types of bacteria were sampled for the monitoring of intestinal and nasal resistance. AMU was recorded on 15 of these farms. AMR rates for 12 antimicrobial agents demonstrated large differences between intensively reared veal calves and more extensively reared dairy and beef cattle. Data analysis showed a strong relation between intensity of AMU and increased resistance. Antimicrobials given orally in low doses and antimicrobials used preventively as group medication promoted the rise of multi-drug resistance in bacteria from the digestive and respiratory system.

The indicator animal-defined daily dosages (ADDD) was used to evaluate AB use for 3 farmer groups and 6 treatment categories in the Netherlands[65]. Large variation in AB use was found between herds, and variation in use among herds decreased during the study period. Reducing AB use and the variation in use were related to changes in management practices, which can enhance AB use awareness. Managing udder health is the main reason for AB use, justifying 68% of all ADDD. Restrictions on CIA drugs were successful in minimizing their use, with a shift to increased use of penicillin.

One study from Romania aimed to determine the presence or absence of AB residues in **dairy** milk, assess the most frequently encountered AB classes and to establish a correlation between positive samples and various milk quality parameters[201]. The research included 360 samples between 2016 and 2017. 11 samples had AB residues above the maximum admissible limit. In positive samples, the number of somatic cells as well as the total number of pathogens increased.

The next 2 sections focus on the specific conditions of mastitis and lameness in **dairy** cattle.

Mastitis

A study from Ireland details on-farm usage of intramammary antimicrobials[78] and highlights positive national progress, particularly with respect to AMU during cow lactation, but also highlights areas for review and further research (e.g. blanket dry cow therapy, BDCT).

A survey of **dairy** farmers on their attitudes, knowledge, and behaviour regarding mastitis before the start of a national Dutch mastitis control program in 2004 was conducted with 204 farmers completing a similar survey in the final year of the program in 2009[76]. Although the average annual bulk milk somatic cell count (BMSCC) remained the same, the farmers' self-reported attitudes, knowledge and behaviour changed significantly.

The social factors influencing farmers' decision-making on the duration of AB treatment of clinical mastitis was explored in the Netherlands and Germany[331]. Extended treatment is perceived as part of the social norm of "being a good farmer." The participants' perception was that mastitis is not treated "thoroughly" if clinical symptoms were still visible at the time of cessation of treatment. Groups with whom the farmer identifies with and regularly communicates with, such as other farmers and the herd veterinarian, confirm the farmer's judgment on extending treatment. This then can influence him/her toward, or away from, socially accepted behaviour.

Despite comparatively strict AB use regulations in Sweden, farmers do not report lack of access to ABs when needed[125]. Structural limitations faced by farmers, rather than lack of information, impose constraints to further limiting AB use in Sweden. This paper draws on the concept of the "good farmer," to interpret findings[125]. Overall stricter and more uniform global regulations on AB use in animal farming could be an effective measure for reducing AB use.

A study in Romania analysed the prevalence of clinical mastitis in a **dairy** farm and the economic implications of this condition over 8 months[202]. Based on the clinical signs, 37% of lactating cows were diagnosed with mastitis and 29 % of these showed relapse. The economic loss due to the compromised lactation and the medical treatment was amounted to almost 25.000 Euro / 8 months. The results of the statistical study performed in this work raised an alarm and highlighted the need of introducing control programs, early diagnosis and mastitis prevention.

A new therapy for mastitis is being developed by *Mastivax* without the need for ABs[348]. Blood of an infected dairy cow is collected, and the immune cells are isolated. The immune cells are then injected into the infected part of the udder. Under laboratory settings it has been demonstrated that the immune cells kill the bacteria causing mastitis. However, this procedure needs further research to investigate effectiveness and treatment frequency.

In France, the **dairy** industry has a National Mastitis Plan to meet the double challenge of market competitiveness and milk quality[35]. The ambition of the national project is to bring together experts in this area, to update, strengthen technical knowledge and provide all the elements for better mastitis control. The mastitis plan contains recent scientific articles, technical information and publications on mastitis. Another part is a toolbox with 'anti-mastitis habits' which can be used on-farm.

A study from Italy established a procedure to identify cows that need to be treated with selective dry cow therapy[44]. SCC from milk test records are a convenient, accurate and certified method. SCC values obtained before drying off or calculated as the average of lactation records can be used. The thresholds of 100,000 cells for primiparous cows and of 200,000 cells for multiparous cows are suggested as an efficient and sustainable decision tool[44].

Reduction in AMU in **dairy** cows with mastitis requires increased diagnostic efforts, as demonstrated in Denmark[130]. The identification of therapy-worthy animals and treatment sensitive cases can contribute to the reduction of AMU by ~50% in treatment of clinical mastitis and ~30% in AB dry cow treatment. Avoidance of wrong decisions with unfavourable long-term effects and related adverse consequences for animal welfare requires systematic udder health monitoring on dairy farms.

The M-team from UGhents' faculty of veterinary medicine (Belgium) provides an action guide with 10 critical points for the attention of the **dairy** farmer to 1) prevent new mastitis infections, 2) shorten the recovery period of infected cows and 3) increase the odds of full clinical and bacteriological cure[246]. The 10 points range from good milking technique to culling of chronically infected cows and breeding for better udder health. Practical tips and relevant information are collected in farmer-friendly language that allow the reader to pinpoint the areas to focus on and how to take corrective action.

An innovative French training program involving participatory classroom training, virtual classes and personalized advice on farm has been evaluated by an exposed/non exposed study on two themes: control of clinical mastitis and implementation of selective dry cow therapy[255]. Exposed farmers significantly improved their knowledge regarding intramammary infections and selective dry-cow therapy compared to non-exposed farmers. Those from the "control of clinical mastitis" group have decreased the use of ABs for mastitis during lactating and dry period and reduced their AB use from 4 to 3.1 days/year/cow.

Extended lactations have been shown to be successful in **goats** and **sheep** in the Netherlands, reducing the number of health problems observed around calving (defined as lactations of 500 days or more). Based on a dataset of 116 organic **dairy** farms, calculations were made to find out to what extent the total milk production per animal was affected by the lower daily milk production and the overall shorter dry period at extended lactations[349]. According to the authors the lowered daily milk production was covered by the increased number of milking days and overall shorter dry period. Theoretically, the overall milk production could even be higher than total milk production collected at mean lactation of 345 days. However good food quality, good management and healthy animals are needed to make extended lactations successful.

Lameness

The UK has an initiative to tackle lameness called the *Healthy Feet Programme*, which provides a checklist to help farmers and vets make the necessary changes to reduce lameness and improve business performance[156]. It recommends to treat certain forms of lameness, such as sole ulcers and white line disease with a foot trim, block and a non-steroidal anti-inflammatory drug, rather than using ABs. For infectious lesions, such as digital dermatitis, ABs may be entirely appropriate. ABs for footbaths are not licensed and are inappropriate.

Sheep

The Sheep Health and Welfare Group in the UK have produced an industry guidance document for veterinarians and farmers on responsible AB use in **sheep**[215]. The report identified the following three areas of concern involving whole flock prophylaxis - control of infectious lameness, prevention of enzootic abortion and treatment of lambs against neonatal bacterial infections. The primary recommendations are to replace, refine and reduce ABs in these target areas.

Anthelmintics must also be used responsibly as part of a farm-specific responsible medicine plan[114]. All prescribers of anthelmintics (to include Vets and Suitably Qualified Persons) should:

- use diagnostic information for each parasite risk period to ensure treatment of only those animals that need it;
- target the drug used to the parasite to be treated;
- treat based on actual body weight;
- understand the interplay of other host species and intermediate host species;
- advise that newly treated animals *should not* be moved immediately onto clean pasture;
- explain and emphasise the importance of quarantining incoming animals, assessing their parasite burden, faecal worm egg counts, and response to treatment;
- investigate suspected cases of resistance and advise on the selection of alternatives from other classes of anthelmintic drugs;
- report suspected cases of lack of efficacy to the relevant authority;
- encourage holistic and integrated preventive strategies.

Pigs

In 2015, the colistin resistance gene (*mcr-1*) was discovered in China. This *mcr-1* gene was then reported in Europe in 2016 in bacteria from farm animals (poultry and pigs). Currently, eight '*mcr*' genes have been reported (*mcr*-1 to 8). In 2016, the European Medicine Agency aimed to reduce the use of colistin in animals in the European Union and a voluntary strategic plan to reduce colistin use in **pigs** was introduced in Spain. A total of 70% of Spanish pig production companies joined the program, representing 80% of Spanish pig production. The impact of these recommendations in the colistin resistance levels in pigs in Spain is reported in this article[231].

Presence of multidrug antimicrobial resistance (multi-AR) in *Salmonella enterica* in **pigs** in Spain was investigated together with association of multi-drug resistance to ceftiofur or tulathromycin treatment during the **pre-weaning** period[236]. Sixty-six *S. enterica* isolates were recovered from five of the eight farms studied. Multi-drug resistance was common, especially for ampicillin, streptomycin, sulphonamides and tetracycline. These ABs are used frequently in veterinary medicine in Spain and, therefore, should be used carefully to minimise the spread of multi-drug resistance.

An AMR monitoring survey shows that **pigs** slaughtered in Romania during the year 2015 were more than 60% colonized with *E. coli* strains resistant to cephalosporins, sulphonamides, tetracyclines and fluoroquinolones[210].

An expert panel analysed the relationship between AMU in the Dutch livestock sector and the prevalence of AMR micro-organisms in livestock[343]. Changes in usage and resistance levels between 2009 and 2014 for most of the commonly used ABs caused the strongest declines in the **pig** (54%) and **broiler** (57%) farming sectors. In most livestock sectors, total and antimicrobial-specific usage levels are clearly associated with antimicrobial-specific resistance levels.

The **pig** industry uses more medication (mg of active ingredient / population correction unit) than other livestock sectors, especially during the **weaning** period when pigs face several challenges and stressors including changes in diet, separation from the sow and mixing of piglets from different litters. These changes cause stress to the animals and compromise their immune system, making them more susceptible to infectious agents. A study from Ireland suggests that the removal of prophylactic in-feed ABs is possible with only minor reductions in productive performance and health which can be addressed by improved husbandry and use of parenteral ABs[487].

Prophylactic use of AB in feed around **weaning** is common on Irish pig farms. Another study from Ireland has shown that removing AB from the feed of weaner pigs had minimal effects on health and welfare indicators[488].

It is assumed that ABs had a transformative effect on livestock production by making it possible to keep larger numbers of animals in smaller spaces without them succumbing to disease. A historical review article argues that their impact has been overstated[305]. It draws on evidence from the veterinary, farming and government literature to demonstrate the significance of other methods devised by vets, farmers etc. of reducing diseases that emerged in association with intensive production systems. These methods predated ABs and evolved alongside them. They understood pig diseases as highly complex interactions between pigs and their environments. Recognition of the roles played by housing, husbandry, nutrition, and pathogens in the production of pig disease suggested multiple possible points of intervention other than ABs. This article challenges existing claims about ABs role in intensive farming and draws attention to other methods of promoting pig health, which may find renewed applications as we move towards a post-AB era.

The use of group medication with ABs in a Danish pig herd was reduced after vaccination against proliferative enteropathy (PE) caused by *Lawsonia intracellularis*[198]. 7900 pigs originating from a single commercial sow herd were vaccinated against *L. intracellularis*, whereas 7756 pigs were kept as non-vaccinated controls. In the vaccinated batches, the consumption of oxytetracycline to treat PE was reduced by 79%, with a significantly lower number of pigs being treated. Vaccination also resulted in a highly significant improvement of average daily weight gain (+ 46 g/day) and carcase weight (+ 1.25 kg) as well as a shortened fattening period (-8 days).

There is growing advocacy for AB-free (ABF) livestock production to minimize the emergence of AB-resistant food-borne pathogens and subsequent human exposure to these treatment-refractory organisms[484]. However, the results from a study in the US indicate that in a PRRSV (Porcine reproductive and respiratory syndrome virus)-endemic setting involving bacterial co-infections, an ABF production strategy may leave pigs at considerable risk of exposure to severe clinical disease and that judicious use of ABs can significantly improve animal health.

A Dutch study revealed interesting differences in AMU in **pigs** following the use of different animal defined daily dosages[72]. Differences in outcomes in an animal species can be attributed to the applied animal defined daily dosage due to differences in authorized indications and dosages, in prescription patterns between farm types, differences in animal (sub) categories and standardized animal weights. This study underlines the urgent need for internationally harmonized units of measurement applicable in monitoring systems for AMU in livestock, such as generic animal defined daily dosages.

An international collaboration aimed to define the daily dose per animal (DDDA) for each active substance and administration route for antimicrobials used in **pig** production and authorized in four European countries, thus allowing cross-country quantification and comparison of AMU data[165]. All veterinary antimicrobial products authorized for use in pigs in Belgium, France, Germany and Sweden were listed per administration route. Four major recommendations are: (i) urgent need for harmonization of authorization and recommended Summary of Product Characteristics (SPC) dosages; (ii) expand the developed preliminary DDDA list to include all authorized veterinary medicinal products in all EU member states and for all (food-producing) animal species; (iii) improved accessibility of country-specific SPC data would be preferable; and (iv) statement of the 'long-acting' duration of a product in the SPC.

GVET is a voluntary, computerized register for all the treatments in **pig** farms in France [15]. It has been active since early 2017 and is run by the French Institute for pig and pork Industry (IFIP). Data collection is available for subtopics like weight categories (**sows, fatteners, weaners and sucklers**) and input is asked about AB treatment (like dosage, date of administration, duration, reasons of treatment, etc.). Analysis is used for farmers and for national and European purposes.

Between January and October 2010, AMU data was collected retrospectively on 50 **closed pig** herds[111] in Belgium. An overall higher use of prophylactic antimicrobial group level therapy was recorded in 2010 compared to 2003. This shift was marked by a partial yet substantial replacement of older, orally-administered compounds by new injectable long-acting products. The most frequently used antimicrobial orally applied to groups of pigs was colistin (30.7%). The most frequently applied injectable antimicrobials were tulathromycin (45.0%), and long acting ceftiofur (40.1%). Injectable products were generally overdosed (79.5%), whereas oral treatments were often under dosed (47.3%). In conclusion, this study shows that preventive group treatment was applied in 98% of the visited herds and often includes the use of critically important and broad-spectrum antimicrobials.

Surveys of representative samples of **pig** farms in France - the INAPORC panels - performed in 2010, 2013 and 2016 aimed to understand the major areas of reduction in AB use over six years[175]. Over the six years, the mean number of treatment days significantly decreased for all age categories of animals. However, for sows the decrease was less marked (-7%) than for suckling piglets (-28%), weaned piglets (-70%) and fatteners (-71%). Other major results included a considerable decrease in the use of critically important ABs premixes and colistin. This did not result in increased use of other digestive ABs or in a massive use of zinc oxide (16% of farms using zinc oxide in 2016).

A Spanish survey describes AMU per production stage in terms of drugs, routes of application, indications, duration and exposed animals in **farrow-to-finish pig** farms in Spain[238]. Information was collected via a questionnaire during the six months prior to face-to-face interviews, completed from April to October 2010, for a total of 49 farms. Results show that the growing stage (from weaning to beginning of finishing) has the highest AMU, feed is the administration route with the highest antimicrobial exposure (because of the high number of exposed animals and longer duration of treatment); and there are large differences in AMU among individual pig farms.

Another large cross-border collaboration aimed to compare AMU for pigs by age category, antimicrobial class and administration route for **pig** herds in four EU countries[185]. The study involved 227 **farrow-to-finish** pig herds in Belgium, France, Germany and Sweden. The Swedish herds had the lowest and the German herds the highest overall use. Most treatments were applied to weaned piglets except in the Swedish herds (with more frequent treatment of suckling piglets). Antimicrobials were most often applied through feed or water except in the Swedish herds where parenteral treatments were most frequent. Aminopenicillins was the antimicrobial class most commonly used. Belgian herd use of third and fourth generation cephalosporins was higher compared to the other countries. In summary, there were large differences in antimicrobial use for pigs between countries, herds and age groups in farrow-to-finish herds of similar size.

Farm-related factors influencing AMU in 60 **farrow-to finish pig** farms in Sweden was investigated and how biosecurity level, farmers' attitudes to AMU and information provided by the herd veterinarian influence AM use under Swedish conditions[317]. There was no significant association between biosecurity and AMU; attitudes to AMU were also not significantly associated with AM use. However, individual characteristics of the farmer were found to be important. Older farmers, females and university-educated farmers used more AM in suckling piglets, and older farmers used more AM in weaners. Larger farms were associated with higher treatment incidence in fatteners.

Under the European Innovation Partnership for Agricultural Productivity and Sustainability (EIP-AGRI) a focus group looked into how to reduce AB use in pig farming[345]. In their report, recommendations and proposals are presented. The group identified three main areas where practical solutions already exist or may be further developed to reduce AB use:

– General enhancement of animal health and welfare to reduce the need for AB use through better biosecurity, management and husbandry, facility design and management, and training of personnel, veterinarians and advisors.

– Specific alternatives to ABs including vaccination, feeding approaches and breeding.

– Changing attitudes, habits and human behaviour (farmers, agri-advisors and veterinarians) and improving the dissemination of information.

Pioneers in pig husbandry in Europe have been successful at reducing antimicrobials without sacrificing productivity or performance[49]. In field studies it has been shown how a multi-stakeholder approach can help achieve goals while reducing their reliance on antimicrobials with 4 take-home messages - Benchmarking optimises success; It is all about prevention; An integrated approach is key; and Plan, do, check and act.

Poultry

Chicken products may be a source of infection with pathogenic *Escherichia coli* strains and may present a zoonotic risk through multi-AB resistance. A total of 30 strains of *E. coli* have been isolated and identified from fresh chicken carcasses harvested at different time intervals over a period of 12 months from different manufacturers in Romania[207]. Strains identified as *E. coli* were tested on 12 antimicrobial substances and showed resistances to multiple ABs. The highest resistance was recorded for erythromycin and doxycycline (96.6%), and the smallest resistance was recorded for gentamicin (10%). *E. coli* strains with multiple AB resistances are one of the main cause of infections in humans and birds.

In a Latvian study, *Campylobacter* species and their AMR in **broiler** chicken production was determined[192]. Resistance to one or more antimicrobials was detected in all 58 isolates identified. A very high proportion of the isolates were resistant to ciprofloxacin and nalidixic acid. Multidrug resistance, which was determined as resistance to three or more unrelated antimicrobials, was detected in 67.2% of the isolates identified.

A further study reports the prevalence and AMR of *Salmonella* isolates from meat in Latvia[193]. A total of 3,152 samples of raw and ready-to-eat (RTE) meats were collected during the official control and in-house control procedures in 2015. The prevalence of *Salmonella* was 0.8%. The highest prevalence (1.5%) of *Salmonella* was found in minced meat and meat preparations, while the lowest (0%) in frozen meat and meat preparations and RTE meats. In total, 62% of *Salmonella* isolates were resistant to at least one antimicrobial.

Antimicrobial susceptibility results from an Estonian study indicate that the use of antimicrobial agents, particularly fluoroquinolones, in **broiler** chicken production has reduced in recent years in Estonia[195]. This can be associated with the policies on restrictive use of antimicrobials implemented by the European Commission in 2006. Resistance to one or more antimicrobials occurred significantly less frequently in the products of Estonian origin than in the products of Latvian and Lithuanian origin available in Estonian retail outlets. It was found that problems caused by the inappropriate use of antimicrobials extend beyond the country from which a food product originates; therefore, the origin of broiler chicken meat may affect the risk involved for the human population.

A further study aimed to determine the AMR of *Escherichia coli* strains from flocks belonging to an integrated consumer **egg** producer[199]. Results indicated presence of resistance to a range of AB agents. In conclusion, even though ABs are used to a lesser extent in chicken flocks for egg consumption compared to poultry meat production, this study found AMR *E. coli* strains and multi-drug resistant strains in egg production as well.

In 2012, a serotype of *Salmonella* (*Salmonella Corvallis*) carrying multi-resistance genetic material was detected in a wild bird in Germany, which researchers then observed had transferred to bacteria in **broiler** chickens[229]. Results in this article indicate that for the future, reduction in AMU must be combined with alternative approaches that target the loss of the resistance gene, in order to slow down the spread of resistance.

Currently, poultry production organizations are committed to defining an indicator for monitoring the responsible use of ABs. The French Poultry Institute (ITAVI), with the French poultry's inter-professional organizations and Anses formalize a professional network to collect computerized data on AB use at farm level (*RefA²vi project*). The network's objective is to produce regular references on AB exposure indicators calculated on the basis of a common method and measured for each poultry species, in particular **turkeys** and **broilers**[506].

In 2014 and 2015, two surveys enabled collection of data from poultry farms in all species and production types. The RefA²vi project has allowed industry professionals to have precise and reliable national references, expressed by one or more indicators in accordance with national and European recommendations[21].

Monitoring consumption of AMU is an indispensable step to have a better knowledge of practices, and consumption levels in order to implement improvement actions and evaluate them. Software for consumption monitoring has been developed by the company DBM[269]. It is an innovative follow-up tool that helps the animal breeder to adopt a sustainable animal health management approach. This software can be used by the breeder and the veterinarian: it measures and assesses joint actions set up to reduce the use of ABs, compares the performance by production, sector or breeder in relation to the results of all livestock monitored and also to reference indicators.

Sanders and Ceva joined forces to launch Indic@Vet: Indic@Vet is an innovative and unique tool for monitoring the consumption of ABs in **rabbit, layer, broiler and pig** farming, developed by the company DBM for Sanders in partnership with the French veterinary laboratory Ceva[17]. Indic@Vet makes it possible to objectify, measure and enhance the actions put in place to reduce AB use, to compare changes by production, sector or breeder, and with French or European benchmarks. Based on drug consumption data and official calculation methods, a follow-up calculation of AB consumption is carried out in order to view the official French and European indicators (mg / kg, ALEA, DDDvet, DCDvet) for each quarter or each batch of animals.

In 2017, the Dutch **turkey** and **broiler** farming sectors managed to substantially reduce their AB use in terms of defined daily doses animal (DDDvet). They achieved reductions of 23.7% and 7.8%, respectively[344].

A French survey aiming to reduce AB use in **turkey** and **duck** farming for **foie gras** production was conducted in 70 breeding lots[265]. This study revealed a lot of risky practices with 31% of the batches receiving at least one treatment, however, the AB Treatment Frequency Indexes were lower than 0.3 (0-21 days and 0-12 weeks); AMU was low. No critical ABs were used to treat the animals during the study. Factors associated with AB treatments focused on hygiene and biosafety precautions, vaccination and water quality.

The social sciences highlight how individual but also organizational, institutional or even market factors, as well as different levels of scale (farms, veterinary practices and territories, public policies and wider society), interact to promote the reduction of AB use in livestock[521].

Flock health plans have evolved from assurance scheme checklists to the foundations of an active partnership between farmer and vet[47]. In the past health plans were often shelved by producers who saw them as a box-ticking exercise, required only to demonstrate that the farm complied with assurance scheme rules. The plan is now often used as a dynamic document that not only sets out procedures but also serves to highlight issues that arise during a production cycle. The components of the plans have also changed – fuelled by the ongoing drive to cut AB use. Topics that are discussed are among others vaccination programmes, biosecurity planning and contingency planning.

In order to understand the human factor on the variability of AMU, a survey was conducted in 2016 with 68 **broiler** breeders in the Brittany and Pays de la Loire (France) regions[273]. The use of antimicrobials, or at least the perception of the level of use by farmers (up or down), is directly related to the perception of their profession (positive or negative). The perception of the poultry profession also seems to be linked to the observance of certain health prevention practices. A positive view of the profession seems to be associated with farmers who tend to reflect on their use of AB treatments.

Poultry farmers of Canada have produced an infographic which describes their AMU strategy to demonstrate responsible use and to reduce use where possible[139]. The Canadian poultry industry has eliminated the preventive use of Category I ABs in 2014 and since then the industry is no longer permitted to use them. Now, they are analysing AMR data, reviewing best management practices, ensuring effective controls of AB use across Canadian farms, educating stakeholders, and researching and sourcing alternative products.

Other species

For breeding **rabbits**, an indicator of sustainability to evaluate the practices of AB supplementation has been created in France[263]. The frequency of AMU was defined as Index of Frequency of AB Treatments (IFTA). The IFTA corresponded to the number of treatments received per animal and day during a reference period. It was declining for the growing rabbits and for the reproductive females. It varied between 0 and 3 (expressed with two decimals to be sensitive to one day of treatment). For 57 breeding units studied, IFTAc (growing rabbits) was 0.86 and IFTAr (breeding rabbits) was 1.44.

Summary

A multi-stakeholder, integrated systems approach has been shown to be a core theme to reduce AMU across livestock production. As demonstrated in the above examples, a combination of monitoring and surveillance of not just AMU but also AMR is necessary to measure progress and target interventions. This can be at the individual and regional farm level, at the veterinary practice and veterinary profession level or wider still at the regional, national and international policy level. International bodies and organisations, such as the OIE and World Health Organisation, often start AMU reduction strategies and frameworks, which then filter down to national Action Plans and initiatives ending with industry acting and forming private-public partnerships. Benchmarking and using the data to add value to herd or flock health management has also been found to be key. Providing more support to farmers and veterinarians either in focus groups or via training has been shown to change behaviour and help reduce AMU. Strategies that focus on animal health and welfare deliver the most sustainable gains in AMU reduction, whether that be vaccinations, biosecurity improvements or improved nutrition and housing. Efforts across nations have resulted in widespread reductions in AMU, with some countries making more progress than others. The links between AMU and AMR show that driving down excessive or unnecessary use is paramount. Certain practices such as in-feed ABs and prophylactic AB use are being phased out across species. The use of data-driven decision-making and harmonised surveillance has been highlighted as a core principle for most countries and regions.

5.10. Prudent use

Highlights

- This chapter concerns prudent use of antimicrobials at farm level, i.e. by farmers and veterinarians. National plans were discussed in the previous chapter on antimicrobial use (AMU) reduction strategies.
- Prudent AMU is important to prevent antimicrobial resistance (AMR) to antibiotics (ABs).
- Veterinarians need to resist pressure from farmers to prescribe ABs, esp. ABs that are critical to treat human patients.
- Selective dry cow therapy (SDCT) means that not all cows are given ABs in the udder at the moment of drying off (as is done in so-called blanket DCT). Cows with a reduced risk of infection (as indicated e.g. by the absence of mastitis in the previous lactation and low somatic cell count (SCC)) are dried off only using a teat sealant.
- In **pigs**, **poultry** and **veal calves** prudent AMU mainly means that vets and farmers must be more reluctant to treat entire batches of animals, and stop the use of ABs as growth promotors or as a routine measure to prevent diseases (e.g. in **weaned pigs**).

What is prudent use?

Prudent use refers to the responsible and thus restricted antimicrobial use (AMU), especially antibiotics (ABs), aiming at limiting the probability of bacteria developing antimicrobial resistance (AMR). Prudent use is often related to AMU reduction strategies (discussed in the previous chapter). These strategies primarily refer to policy measures taken at regional or national level, whereas prudent use mainly concerns application at farm level, as applied by farmers and veterinarians.

How does prudent use help reduce antimicrobial resistance?

Prudent use implies using less ABs and using ABs in more effective and specific ways, so as to limit the probability of development of AMR, e.g. by using ABs that target the specific problem, rather than using broad-spectrum ABs.

Why is prudent use important?

Prudent AMU plays an important role in the prevention of AMR, which represents a concern for the health of both humans and animals, being associated with morbidity, mortality and economic losses in livestock farming systems[372]. Moreover, it is important to limit the application of ABs considered critical for the treatment of human diseases in livestock farming and give priority to its application in human medicine.

The overuse and misuse of antimicrobial products has dramatically contributed to the emergence and spread of AMR organisms, which pose an extraordinary threat to human and animal health, and to the global ecosystem[443].

AMR has been a public health threat globally, with millions of lives lost due to AMR infections each year[372]. The cases of AMR continue to increase and cause devastating effects to both humans and animals (incl. high morbidity, mortality and staggering economic losses to livestock producers). The main factor for AMR in this industry is the eagerness of AMU in livestock producers to promote animal growth and prevent diseases. AMR in farm animals can jeopardize human health due to the dissemination of AMR pathogens to humans via consumption of infected products, like meat or dairy, or direct contact with infected animals.

At the current rate of unrestricted AMU, AMR will be expedited and soon we will run out of effective treatment for common infections. The World Health Organization (WHO) has issued guidelines for medically important AMU in animals to mitigate the adverse consequences of AMR on humans[372].

The widespread AMU in animals has caused concerns about the growing risk for AMR. In 2011-2012 AB consumption was higher in animals than in humans according to the of EFSA (European Food Safety Agency), ECDC (European Centre for Disease Prevention and Control) and EMA (European Medicines Agency). Both in humans and animals, positive associations between the consumption of ABs and resistant bacteria have been observed. Therefore, responsible AMU in humans and animals should be promoted[409].

The spread of AMR requires new solutions for both disease prevention and disease treatment. As the rate of development of new ABs has severely declined, alternatives must be considered in both animal agriculture and human medicine. Products for disease prevention may differ from those for disease treatment. For example, the modulation of the gut microbial community, either through feed additives or faecal transplantation, could be a promising way to prevent certain diseases; for disease treatment, non-AB approaches include phage therapy, phage lysins, bacteriocins, and predatory bacteria. Interestingly, several of these methods augment AB efficacy by improving bacterial killing and decreasing AMR. Because bacteria can ultimately develop resistance to almost any therapeutic agent, it is important to continue to use both ABs and their alternatives judiciously[410].

What is interesting and worthwhile knowing about prudent use?

This section discusses findings reported in the DISARM database on prudent use. Subsections deal with general issues **across species, cattle, pigs, poultry, sheep and goats**, and publications concerning **multiple species**. Subsections concern specific compounds and solutions for prudent AMU, and publications that specifically address veterinarians.

General (across species)

In order to understand the importance of prudent use and how it can be achieved, it is relevant to fully comprehend the general aspects of AB resistance such as microbiological versus clinical resistance, intrinsic versus acquired resistance, resistance mechanisms and transfer of resistance. This information is also available in our database[409].

A compilation of instruments was made containing implementable standards for governments or other actors regarding AMU. This excludes action plans only stating intentions and international standards adopted by private entities and business associations[373].

In 2018, the World Veterinary Association (WVA) and the World Organisation for Animal Health (OIE) conducted a survey to create a global repository of guidelines for responsible AMU in animals. It contains 120 guidelines, action plans and promotional material for vets and other health professionals[184].

Another useful manual in our database is a guide to prudent AB use, which includes medically efficient ways to avoid a loss in productivity. Strategies mentioned in this guide that can be effective for a prudent use of AB include the following: a) Phasing out AB use as growth promoters and avoiding regular preventive AB use; b) Avoiding use of the Highest

Priority Critically Important Antimicrobials (CIAs) for human medicine in animals and adhering to the OIE List of Antimicrobials of Veterinary Importance; c) Only using ABs based on a diagnosis by a vet or other animal-health professional and only for authorized indications; d) Striving for individual treatment of animals with the correct dose and duration and avoiding using group AB treatments except for **poultry** flocks, especially via feed. e) Using only quality-assured pharmaceuticals and always consulting an animal health professional before use; f) Disposing of unused and expired ABs in a proper way[500].

Some countries have issued specific guidelines. In Germany, compulsory guidelines for responsible AMU and AMR were published in 2000 focussing on an exact diagnosis, using AB with a narrow spectrum of activity, high safety margin and good tissue penetration, avoidance of critically important ABs, and adherence to dosage prescriptions (no under/prolonged dosing). Deviations from the guidelines must be justified and recorded. AMU (esp. medicated feed) resulted in a reduction in treatment days/animal from 31.6 before the guidelines to 13.6 days by 2002. The use of chlortetracycline decreased from 76% of total ABs to 14.7% at the end of the study. Thus, the responsible AMU guidelines have been an important tool to reduce AMU[56].

A benchmark indicator for AMU on farms in the Netherlands was introduced in 2011. The Dutch law prescribes that each farm is linked to one specific veterinarian, and therefore analysis of defined daily dosages animal per farm (DDDAF) can be done for all farms served by the same veterinarian. The benchmark indicator estimates the likelihood of exceeding the farm action benchmark threshold for all farms for which the veterinarian is the contracted veterinarian. The benchmark for veterinarians stimulates self-regulation of the veterinarians[308]. In the Netherlands AMU is monitored via the Dutch Veterinary Medicine Authority which makes annual reports (Neth-map/MARAN) on the national trend regarding AMU.

Australia's vets and livestock industries have long worked on AMR, in close cooperation with the government. Australia’s First National Antimicrobial Resistance Strategy 2015-19 (National Strategy) had 7 objectives. The second objective concerned **pigs** and required antimicrobial stewardship to ensure appropriate prescribing, dispensing and administering of antimicrobials. These guidelines are specific for Australian vets[183].

The DISARM database also contains several entries such as: a) the national AMR plan in Spain[451, 452] (a Spanish guide for AB use in line with integrated agriculture[453]); b) an information sheet to raise awareness about biosecurity and AB use[457]; c) a document describing principles for AB reduction through prevention, correct and early detection and precise treatment[454] (both in Spanish); d) a Code of Good Practice regarding the responsible prescribing and use of AB in farm animals for Irish farmers and vets [405].

An additional aspect of prudent use is soil management. Soil is one of the biggest reservoirs of microbial diversity and is vital for agricultural purposes. Soil is a favourable environment for the development of AMR, due to its high complexity and ongoing competition between microorganisms. In this study comparing the soil microbiota and its resistome in conventional and organic farming systems (inorganic fertilizers and pesticides vs. organic manure and no chemical pest management), no major differences were found among the main phyla of bacteria between the two farming styles with similar soil structure and pH[424].

Specific compounds and solutions to prudent AMU

Several articles have provided an overview on alternatives for AB (see also Section 5.8 on Specific Alternatives).

Macrolides and lincosamides (ML) are important antimicrobials for treatment of **pigs** and **cattle**, esp. major swine gastrointestinal and respiratory infections and bovine respiratory disease, respectively. There are still many old products containing ML in the EU, and their dosing regimens need to be updated. Acquired AMR to ML has emerged, esp. in *Brachyspira* and it is mainly due to in-feed medications and long-acting injections resulting in low concentrations of the active substance for long periods. Prudent use of ML antimicrobials is crucial to maintain the efficacy of these important therapeutic drugs[75].

Colistin is still often used outside the EU as an AB to improve animal performance and health. Hops (*Humulus Lupulus L.*), known for its effects on microbial cell structures, can represent an alternative to the use of colistin. Hop extracts (β-acids) in **piglet** feed can improve performance (daily weight gain, daily feed conversion ratio), mainly by improving nutrient digestibility and intestinal health[381].

One entry in our database investigated products having the potential to be used in livestock production ranging from plant extracts to prebiotic functional feed ingredients. All functional feed ingredients showed potential in improving performance and health. There was no clear preference for a particular functional feed ingredient, and ultimately it was concluded the final selection would mainly depend on farmer expectations and commercial price setting[374].

In yet another overview of plant and herb extracts, AB substitutes in livestock production were revised by experts in the field. A portfolio of blends was proposed and the potential of traditional Chinese medicine plants in improving livestock performance and health was also assessed. Not all plant extracts are currently accepted in European legislation. The results of the paper indicate that, in order to clearly define the beneficial effects of essential oils and aromatic herbs, further research and development in this field should be encouraged[375].

Another article looking at the effect of plant-based essential oils against important microbial pathogens, such as *E. coli O157:H7, Salmonella Typhimurium, Staphylococcus aureus* and *Listeria monocytogenes* showed positive results, when these infections were treated in livestock production. However, most plant-based essential oils were tested *in vitro* as pure compounds, and they still need to be evaluated on cost-effectiveness[379].

A study looked at the effect of plant-based alkaloids on *Salmonella.* These alkaloids can be transformed into quaternary benzo(c)phenanthridine alkaloids. The main mode of action is based on maintaining gastrointestinal integrity, blocking *Salmonella* and other pathogens from entering the bloodstream and causing infection. The legal status of the quaternary benzo(c)phenanthridine alkaloids should be discussed, as it may open up innovation in food quality and safety[380].

Other studied alternatives are Eucalyptus-derived medium-chain fatty acids. These can be a promising substitute for ABs, since these fatty acids are not obtained from palm kernel oil, which is also not sustainable. Processing of the medium-chain fatty acids into micro-encapsulated functional feed ingredients improves daily performance and health of animals[377].

Our database also reports the results of allicin in AB-free farming. Like other essential oils, allicin is classified as a functional feed ingredient in livestock production, promoting health and performance. Allicin also reduces manure odour, making farming more accepted for the general public, and improving working conditions for farmers due to a reduction in the attraction of insects (e.g. flies)[378].

Finally, passive immunisation by administration of antibodies (immunoglobulins) has been known for more than one hundred years as a very efficient means of obtaining immediate, short-lived protection against infection and/or against the disease-causing effects of toxins from microbial pathogens and other sources. Examples of passive immunisation in the modern production of **pigs, cattle, sheep, goat, poultry and fish** were reviewed showing that passive immunisation could have a clear role in modern livestock production to control infectious diseases, particularly when highly efficient, relatively low-cost immunoglobulin products were available[411].

Regarding veterinarians

Increased AMR placed a considerable societal duty on vets to act as stewards with respect to prudent AMU. The College of Veterinarians in Ontario expects vets to assume an active leadership role by ensuring their understanding of the need for AMU oversight, the existing government directives, the evolving science related to pharmaceuticals and the most appropriate AMU in specific species. Vets must only prescribe antimicrobial drugs within a veterinarian-client-patient relationship and where strong clinical evidence demonstrates a medical need[113].

In the Netherlands, the Dutch veterinary association provided bovine practitioners with advices dealing with the reduction of AMU during the dry period of dairy **cows** (KNMvD, 2014) in this first veterinary guideline (in Dutch)[286].

Veterinarians are encouraged to check the updated scientific advice made by EMA on the categorisation of ABs when prescribing these medicines for animals in their care. This categorisation can also be used to prepare treatment guidelines. ABs were ranked based on public health risks of AMR and the veterinary need to use them. The update included the list published by the WHO on critically important antimicrobials (CIA), i.e. those of most relevance for human health, and the impact of the route of administration on AMR. The classification comprises four categories: Avoid, Restrict, Caution and Prudence[182].

Category A: These medicines may not be used in food-producing animals and may be given to individual companion animals only under exceptional circumstances. Category B: The use of these medicines in animals should be restricted to mitigate the risk to public health. Category C: These ABs should only be used when there are no antimicrobial substances in

Category D that would be clinically effective. Category D: These ABs can be used in animals in a prudent manner[256].

Research conducted by studying the views of veterinarians in the Netherlands and Flanders (n=174 and n =437, respectively) suggest that most AMU is related to suboptimal climatic conditions. One the one hand, Flemish vets reported that insufficient biosecurity measures and farmers' mentality are important factors related to AMU. On the other hand, the Dutch vets ranked insufficient passive transfer of immunoglobulins in young animals and economic considerations of farmers as major causes linked to AMU. Most Dutch vets, but only about 33% of Flemish vets supported the policy to halve veterinary AMU. Improved housing, climate, biosecurity and control of specific infectious diseases were therefore considered important in reducing AMU[174].

Interviews with 11 Dutch farm veterinarians indicated conflicts of interests related to animal suffering, farmer finances, risk avoidance, limited skills, lack of farmer compliance, public health and personal beliefs regarding AMR. Veterinary attitudes, advisory skills and tools to deal with pressure from farmers should be developed, together with policies supporting a more independent animal-health consultancy role[50].

In some cases, veterinarians may decide to prescribe antimicrobials to **sheep** and **beef** farmers without a clinical consultation. In order to investigate what drove certain individuals to prescribe without consultation, veterinarians were presented with eight scenarios where a farmer would ask for antimicrobials. Case type, farmer relationship with a vet, influence of other veterinarians in the practice, time pressure, habit, farmer's willingness to pay and confidence in the farmer were significant factors in the decision to prescribe. Confidence in the farmer was the variable with the highest influence. Agreeableness personality score, region of veterinary practice and presence of a small animal department had a significant influence on the decision to prescribe. This research indicates that these factors could be considered in targeted interventions in farm animal veterinary practice for improved antimicrobial stewardship[312].

Veterinary students' perceptions, attitudes and knowledge about antimicrobial stewardship and biosecurity could also have an effect on the overall prescription behaviour. A questionnaire for students expected to graduate in 2017 or 2018 in all Australian veterinary schools indicated that many students were unaware of the high importance of some veterinary drugs to human medicine, specifically enrofloxacin and cefovecin. Less than 10% of students would use appropriate personal protective equipment in scenarios suggestive of Q fever or psittacosis. This indicates that there is a need for harmonization of preclinical and clinical teaching, and that biosecurity and antimicrobial stewardship must be further emphasised [446].

(Dairy) cattle

AMU in **dairy cattle** can be reduced by a combined intervention scheme based on: (1) timely clinical inspections, (2) the assessment of animal-based welfare parameters, and (3) the use of predictive laboratory tests, e.g. immunology and chemistry tests to predict production diseases. Many antimicrobials prescribed for chronic diseases are not justified after a cost/benefit analysis. AMU may not lead to greater cure rates for some forms of mastitis.

Lastly, a substantial reduction of AMU in dairy farms can be achieved through the proper use of immunomodulators[304].

Monitoring of AMU has also been part of a national **cattle** health surveillance system (CHSS) in the Netherlands, which includes several surveillance components. These components are: enhanced passive reporting, diagnostic test results and post-mortem exams, random surveys for prevalence estimation of endemic diseases and quarterly data analysis (Trend Analysis Surveillance Component, TASC) to monitor trends and developments in cattle health using routine census data. Key indicators are mortality, fertility, udder health and AMU. The TASC allows visualizing trends in time, it can be used to support or highlight signals detected in the other surveillance components, and it can provide warnings or initiate policy changes[103].

A practical guide to avoid milk AB residues (poster from the BCVA, British **Cattle** Veterinary Association) recommends that unless advised by a vet, farmers should follow the manufacturer's instructions for treatments and withholding time. If in doubt, farmers should consult the vet, who may recommend an AB residue test. Farmers must ensure that milk is clear of Abs, and this is part of the milk-delivery contract. In addition, farmers should follow the data sheet and veterinarian's advice on all treatments. The utilization of different treatments (combinations of products, or a different dose, frequency or prolong treatment) can affect withdrawal times. According to the guide, a milk-withdrawal period of at least 7 milk be applied, and the milk should be tested before being allowed in the tank[115].

Another recommendation regarding prudent use is the US drug-residue prevention manual. This publication concerns administering, recording and storing drugs on **dairy** farms, including an 8-step plan for keeping useful medicine records, comprehensive drug residue testing and lists of licensed drugs and withdrawal periods (in the US). Additionally, there are top tips for producers to reduce AB residues and checklists for when treating animals, e.g. read the product label and consult your veterinarian before administering, use a clean injection site and a sterile needle for all injections, discard milk from all four quarters even when treating only one quarter with an intramammary (IM) tube, make sure that any procedure used to divert milk from treated **cows** cannot accidentally send contaminated milk into the pipeline, train employees on proper injection techniques and do not go back into the vaccine bottle with a needle once it has been used for anything else[120].

Another way of fostering prudent use and AMU awareness can be done through the farmer field school (FFS). The FFS is a concept for farmers' learning, knowledge exchange and empowerment. A Danish research project focuses on explicit non-AB strategies involving farmers who have actively expressed an interest in phasing out ABs. A Danish FFS approach was named "stable schools". A facilitator wrote the meeting agenda together with the host farmer, directed the meeting and wrote the minutes. Identifying problems and solutions for complex farming situations were the focus of all groups. This article describes the experiences of 4 stable school groups for Danish organic **dairy** farming[332].

A study in Western France evaluated an innovative training program involving classroom training, virtual classrooms and individual support of **dairy** farmers. Two groups addressed two themes:

(1) Mastitis prevention during lactation for herds with frequent cases of clinical mastitis;

(2) Selective instead of blanket dry cow therapy (BDCT) for herds with good udder health.

Some farmers appreciated the virtual classroom training because it was flexible and less time consuming. However, connection or computing problems made the participation in the virtual classrooms difficult. For the "prevention" group, results also showed limited improvement in knowledge, perception and practices of farmers. Both exposed and non-exposed farmers improved their practices of AB use at dry-off[355].

Several organizations have also provided practical materials to promote prudent AMU. An entry in the DISARM database explains AMU in **dairy cattle** and recent approaches and challenges on AMR[372], and there is a video on milking (in Danish)[16]. A presentation describes the 4 ways Sweden has cut AB use on **dairy** farms: removing unnecessary AB use, minimizing need and preventing spread, optimising use when needed, and monitoring use and resistance[393]. Another publication focusses on vaccination of **dairy** cows against *E Coli* mastitis[416], and a Spanish information sheet is available on responsible AMU for (young) **cattle** vets[466].

Finally, the effect of AMU on **dairy and beef** products has been considered extensively, but AB residuals into soil and water environments is less regulated and studied. Interviews with 27 dairy farmers in central New York showed that farmers extensively considered the transfer of AB into milk and beef, while consideration of AB residues into manure was less common, and no farmers discussed AB transport from carcasses into soil from on-farm animal mortality. Farmers highlighted decisions that reduce AB environmental loads through disease prevention, non-AB treatments, and culturing bacterial samples before AB treatment. Farmers did not cite reduction of environmental AB loads as a driver of their waste management decisions. Farmers perceived AB usage was already minimized, suggesting future environmental AB contamination mitigation strategies should focus on waste management pathways[499].

Selective dry cow therapy

A UK-based responsible dry **cow** management guide describes that intramammary infection status is used to decide on selective dry cow therapy (SDCT). An elevated somatic cell count (SSC >200,000 cells per ml) indicates infection. It is recommended to use more than one SSC to obtain a reliable result on intramammary infection status (at least three and preferably for the whole lactation) and a good udder/teat status to lower the risk of infections. Internal teat sealants can be used alone when cows had no clinical cases of mastitis in the last lactation, and when the last 3 SSCs have been <200,000 cells per ml. Other tests, e.g. conductivity tests, may also be used to detect subclinical mastitis. Finally, whatever product is used at drying off, an aseptic technique is essential[117].

Until recently, blanket dry cow treatment (BDCT, i.e. always using AB) has been the norm, and therefore the change to SDCT was expected to be a challenge. SDCT was adopted by ~75% of study farms (in a publication from 2016). Four factors related to farmers' mindset were associated with the likelihood to adopt SDCT: costs of SDCT, uncertainty over recovery without AB, lack of concern about potential negative consequences and use of internal teat sealants. Farmers were generally positive about reducing AMU[63].

SDCT in cows with low SCC at the last milk recording before drying off was studied in various Dutch **dairy** herds. The incidence rate of clinical mastitis (CM) was found to be 1.7 times higher in quarters dried off without AB in comparison to quarters dried off with AB. *S. uberis* was the main pathogen causing CM in both udders with and without dry cow therapy (DCT). SCC at calving and at 14 d in milk was significantly higher in quarters dried off without AB (versus with AB). SDCT significantly increased the incidence of CM and SCC. The decrease in AB use by drying off quarters without DCT was not compensated by an increase in AB use for treating CM. Total AB use related to mastitis was reduced by 85% in these quarters[57].

Udder health, AMU and herd economics were studied in one herd in the Netherlands during the dry period and the first 100 d of lactation using 8 different scenarios. These scenarios, which included a BDCT scenario, were used to select cows for DCT based on cow-level SCC at the last milk recording before drying off, for first and later dry periods. CM varied from 11.6 to 14.5 cases per 10,000 cow-days at risk in the different scenarios, and the prevalence of subclinical mastitis varied from 38.8% in scenario 1 (BDCT) to 48.3% in scenario 8 (where the least AB was used). Total AMU varied from 1.27 (scenario 8) to 3.15 (BDCT, scenario 1) Animal Daily Doses, leading to a 60% reduction in AMU for scenario 8 compared with BDCT. The total costs for each of the scenarios was similar. The effect of selective DCT on udder health, AMU and herd economics was influenced by SCC used to select cows. The greatest reduction in AMU was achieved under scenario 8[58].

Another aspect of prudent use concerns the behaviour of the different actors in a farm. Dutch veterinarians' attitudes toward reduced AMU and SDCT were positive. Most think they can still be a good veterinarian when they prescribe less ABs. Veterinarians progressively promoted SDCT at the start of 2013. Veterinarians with a favourable attitude mentioned positive aspects of SDCT, such as an increased awareness of AMU amongst farmers, improving animal health, reducing AMR, and a chance to add value for the farmer. These positive aspects were mentioned more often by these veterinarians compared to veterinarians with a less favourable view on SDCT, who mentioned negative aspects like a higher risk of sick cows and feeling pressured to follow the rules. Given their influence on farmers, veterinarians need specific attention to promote programs to engage farmers in responsible AMU and SDCT [59].

Another study looking at the social aspects of prudent use was conducted in France. A survey of 51 **dairy** farms showed that the advisory relationships between farmers, farm advisors and veterinarians influenced the implementation of SDCT. However, these relationships had very little effect on the use of alternative medicines by farmers, who were more willing to experiment with using alternative medicines than their advisors. The dairy farming system had very little influence on AMU: some misuse of AB was found whatever the farming system. Systematic BDCT was also a widespread habit in all dairy farming systems except organic. The use of alternative medicines was common in all systems[354].

Also in France the current dry-cow practices of **dairy** farmers and their needs have been investigated. An analysis of the national database, which includes information collected from 2,914,921 dry cows between 2015 and 2017, was performed and two surveys were conducted. The first online survey involved 130 advisors from the main French dairy regions including 73.8% advisers, 17.5% team supervisors and 8.7% veterinarians. The second on-farm survey involved 79 farmers in Western and Eastern France. The median duration of the dry period was 63 days. The median milk production before drying off was 16.9 kg/d, with 9% of the cows producing over 25 kg/d. In 2017, on average 12% new infections occurred, and the recovery index was 77%. Large variation between farms seemed linked more to herd characteristics rather than to individual characteristics, e.g. milk production, SCC and dry period. Drying off and dry period practices are very diverse: five major treatment protocols to dry off were used. In addition, 78% of farmers use an AB for drying off, and 50% practice SDCT. Similarly, 138 feeding programs were used, i.e. 1.7 per farm on average (79 farms in total). Udder infections and milk fever were the 2 main peripartum problems cited by 72% and 54% of farmers, respectively. The concerns of advisors related primarily to: (i) feeding management (21%), treatment strategy (20%) and the decrease in milk production (19%) before dying off, (ii) dry-off protocols on the day of dry-off (47%) and (iii) feeding and preparation for calving (39%). Farmers expressed similar themes, but requested a different type of information. Farmers requested more protocols whereas advisors asked for more basic understanding[496].

In Flanders, The tool “Selectief droogzetten in Vlaanderen” [selective drying-off in Flanders] provides a flowchart to use SDCT to facilitate prudent use. Farm determinants include: attitude towards SDCT, good drying-off protocol in place, absence of *Streptococcus agalactiae* from the herd, recording of milk production, and bulk milk SCC (<250,000 cells per ml). The determinants at cow level include clinical mastitis, production level at drying off, data on the last three SCCs before drying-off and specific thresholds for the SCC per cow depending on parity and the 6 monthly geometric average of the bulk SCC of the herd[245].

SDCT includes the option of beginning to dry some **cows** off selectively, i.e. without AB and only with teat sealant. SDCT may reduce AB use, mastitis and medicine costs compared to BDCT (AB tube and teat sealant). A checklist made by Zoetis, a pharmaceutical company, provides a step by step approach to start SDCT[151]. A Zoetis' farmer guide helps farmers using AB in DCT and teat sealant to ‘clean up’ any existing udder infections and seal the teat to prevent new infections until calving. The guide covers methods to observe strict hygiene when preparing the teat and how to insert the tube[144]. Zoetis also has a photo guide or checklist to prepare a **cow**’s udder and teats using a teat sealant, covering basic hygiene and tube insertion technique to minimise the spread of infection[146].

Pigs

The French **pig** sector and national authorities have focused on reduced AMU since 2010. A retrospective study on a representative sample of at least 150 farms in the period 2010-2013 showed a strong and significant decrease in AMU, in compliance with the national target of 25% reduction by 2017. French **pig** farmers were very aware of AMU objectives, and this has resulted in a restriction on the use of 3rd and 4th generation cephalosporins since 2012[106].

Sampling of sixty **sows** and 180 of their **piglets** from three herds in Belgium showed that *E.coli* AMR in piglets was influenced by AMU in sows and piglets, and by the sow resistance level. Sows act as a reservoir for their newborns, and AMU in sows during lactation is a risk factor for the persistence of *E. coli* AMR for the sows and the piglets. AMU in piglets also leads to increased AMR in the piglets during lactation[110].

Regarding AMU perception, a survey of **pig** farmers (N = 281) in Belgium, France, Germany, Sweden and Switzerland showed farmers generally believe their own AMU is lower than that of their fellow countrymen and to use less or equal amounts of antimicrobials compared to farmers from other countries. Farmers were significantly more worried about financial or legal issues than about AMR. Further, farmers who worried about AMR and estimated their AMU as lower than their fellow countrymen, perceived more impact from policy measures on AMU reduction, such as financial rewards for farmers who were able to keep their AMU under a certain threshold and governmental or sector organisations' surveillance and monitoring of farmers' AMU. The same policy measures can be applied to reduce AMU in pig farming in all five countries. Moreover, it is important to increase pig farmers' awareness of the threat of AMR and its relation to AMU not only because pig farmers were not worried about AMU, but also because it affected farmers' perception of policy measures to reduce AMU[189].

Still on the topic of perception around AMU, **pig** farmers' in Belgium, France, Germany and Sweden who used more antimicrobials also estimated their own usage as higher. Farmers perceived many benefits but few risks of AMU in pig farming. Cross-country differences in farmers' perceptions were relatively small. The promotion of prudent AMU should focus on the structural differences in pig farming and veterinary medicine among countries. In addition, interventions aiming at reducing AMU should increase farmers' awareness of the risks of extensive AMU[190].

In a survey in Belgium, Denmark, France, Germany, Sweden and Switzerland, pig farmers (n=1,294) and veterinarians (n=334) had similar perceptions of the risks and benefits of AMU. Veterinarians were more optimistic than pig farmers about the reduction of AMU in pig farming. Farmers believed that their efficacy over AMU reduction was relatively high. Farmers' intention to reduce AMU and veterinarians' self-reported reduction behaviours were connected with factors concerning the feasibility of reducing AMU. To promote prudent AMU, pig farmers should learn and experience how to reduce AMU by applying alternative measures, whereas veterinarians should strengthen their advisory role and competencies to support and educate farmers[191].

A separate publication from Germany describes the perceptions and attitudes towards AMR among general practitioners, hospital physicians, veterinarians, **pig** farmers and the general public. Farmers were predominantly satisfied with existing solutions. Farmers had three times better basic knowledge of AMR and knew twice as many people with multiple AMR problems than the general public. They also received information on AMRR more often from their vets than patients did from their doctors. These results can help tailor future interventions, promote mutual understanding and thus support the One Health approach[358].

Vaccination is a strategy to prevent diseases and to minimise the need for AMU. Data on Danish **finisher pig** herds (n=40–62) collected over a 4-year period showed that vaccination against porcine reproductive and respiratory syndrome (PRRS) and higher AMU for finisher pigs were associated with increased lean meat percentage. Vaccination against porcine circovirus 2 (PCV2), PRRS and *a*ctinobacillus pleuropneumoniae (APP) were associated with higher levels of AMU, and vaccination against *Lawsonia intracellularis* was associated with a higher AMU-ratio (proportion of parenteral AMU treatments out of all treatments). This may be explained by some farmers preferring to take action soon after observing disease problems[197].

The *Yellow Card intervention*, enforced since 2010 on AMU in Danish **pig** production, resulted in a 25% decline in the total AMU per pig produced between 2009 and 2011. The decrease was observed in both sows and piglets (31%), weaners (34%) and finishers (19%). Reduced AMU for oral use in weaners and finishers explained 76% of the total reduction. In 2012, the overall AMU increased by 10%, as a result of slight changes of prescription patterns. At herd level, the decline and subsequent increase was mainly related to changes in the number of herds receiving regular monthly prescriptions. The steep decrease in AMU in the Danish pig production was temporally related with the announcement and introduction of the Yellow Card intervention[212].

Awareness of bacterial AMR in humans and veterinary medicine has raised concerns over antimicrobial overuse. AMU on farms has been challenged because of the perceived risk of transfer of resistant pathogens from animals to humans. Drivers and motivators behind AMU by veterinarians and farmers in the UK **pig** industry were examined in focus groups. Both vets and farmers considered health status to be key in AMU on farms. 'External pressure', e.g. from clients, legislation and public perception, strongly influenced vets' prescribing behaviour, whereas farmers considered issues related to farming systems and management to be greater drivers of AMU. Vets reflected that legislative decisions are driven by political pressure and are not always supported by scientific evidence. Most participants did not think that AMR had affected the health and welfare of their livestock and considered this to be more problematic for others. There was an overwhelming opinion among vets and farmers that they considered themselves to use antimicrobials responsibly, and in the case of the farmers, they considered their respective vets to also be responsible. Production costs were cited by both the farmer and vet focus groups as being a major factor in AMU. Farmers considered their veterinarian to be the most trusted source of information on antimicrobials and not advertisements[337].

In a longitudinal study on 36 conventional Dutch **pig** farms visited between 2011 and 2013 the number of farms with *extended-spectrum beta-lactamase-producing Escherichia coli* (*ESBL-E. coli)* carrying pigs decreased from 16 to 10, and the prevalence of ESBL-*E. coli* positive pig samples decreased from 27 to 13%. ESBL-*E. coli* presence was unrelated to AMU but was strongly affected by cephalosporin use at the farm. Improved biosecurity measures are associated with a lower probability of ESBL positive farms, e.g. a hygiene lock and professional pest control[54].

A research paper described the positive effects of Brazilian red pepper on performance, diarrhoea and gut health of **weanling pigs**[376].

Several countries have issued guidelines to facilitate prudent AMU.

The Australian **pig** veterinarians have specific guidelines as a handy 'go-to' resource of contemporary knowledge on AMR[417].

Spanish organizations have prepared a collection of information sheets for pigs, e.g. to raise awareness about prudent AMU[455], about biosecurity and herd health management[455] (in Spanish), external biosecurity, herd health improvement and the need to reduce ABs[456] , AB use in **pigs**[458,459], slaughter[460], live transport[461] and Salmonella[462].

The Ministry of Agriculture, Fisheries and Food of Spain also has an information sheet on **pigs** related to the Ministry's strategic plan regarding biosecurity[463].

Several information sheets are also available in French, e.g. a VETresponsable's AMU guide for (young) vets[465], and an information sheet to calculate AMU in mg/PCU and compare with other farms anonymously[468].

Poultry

Currently, poultry production organizations are committed to defining an indicator to monitor the prudent use of AB. That is why, the French Poultry Institute (ITAVI), with the French poultry's inter-professional organizations and Anses formalised a professional network to collect computerized data on AMU at farm level (*RefA²vi project*). In 2014 and 2015, two surveys collected data to choose, together with the professional actors, the more pertinent indicators for all poultry production sectors. The computerized data on AB use recorded by the poultry production organizations will be sent to CIPC-CIDEF-CICAR (interprofessional committees for **turkey**, **chickens** and **ducks** respectively) for anonymization and aggregation, in order to send them to ITAVI to calculate the exposure indicators following a proven method defined together with Anses. ITAVI will support the network, e.g. with a newsletter and the organization of steering-committee meetings[508].

Prevention and control of *Campylobacter* in **poultry** flocks are important strategies to control human campylobacteriosis. A critical review showed that many studies had a poor design, sampling and statistical analysis. Biosecurity on conventional broiler farms can often be enhanced and this should contribute to the reduction of flock colonization with *campylobacter*. However, non-biosecurity-based approaches will also be required in the future to maximize the reduction of *Campylobacter*-positive flocks[104].

Spanish organizations have prepared a collection of information sheets on biosecurity and AMU guide for young veterinarians[466], and five factsheets are available about prudent AB use for French **poultry** farmers[505].

Sheep and goats

Veterinary surgeons and **sheep** farmers can work together to deal with three specific disease-management issues – infectious lameness, enzootic abortion of ewes and neonatal bacterial infections – by replacing, refining and reducing AMU, based on guidelines drawn up by the UK Sheep Veterinary Society[313].

Results of a s survey conducted among 46 **dairy sheep** farmers in France, and focussing on pathologies and AMU in young lambs, showed that both feeding and housing conditions are essential to prevent health problems. AMU was perceived as punctual and within reason. The main farming practices which can reduce AMU in lambs are the following : • A balanced diet, including mineral feed, throughout gestation and as a preparation for lambing; • The lambing hutting, the creation of the mother-young bond and the assurance of an important and fast colostrum intake; • Homogeneous batches of lambs in terms of age and weight; • Good environmental conditions in the building and sufficient litter[359]. Results of a survey conducted among 45 **sheep-meat** farmers with a focus on pathologies and AMU in young lambs in France showed the importance of prevention of health problems. ABs were perceived as essential and used sparingly. Frequent pathologies included arthritis, *pasteurellosis* and *enterotoxemia*. Reduced AMU depended on: • Animal surveillance; • The lambing pen and monitoring of colostrum feeding; • Hygiene and care for lambs: disinfection of the navel, tail and ear tags; • A balanced diet throughout the year[360].

In another survey conducted in France among 46 **goat** farmers on pathologies and AMU showed the importance of surveillance and prevention, e.g. being attentive to the first signs of disease and respond to them quickly. Practices most used to reduce AMU in kids are: • Surveillance and care of the animals; • Early feeding of colostrum; • Feed; • Good environmental conditions[361].

Prudent AMU in **lambs and kids** follows a set of prescription and administration rules, described in a French booklet. Lamb and kid rearing aims to limit the occurrence and spread of infections within the herd. Surveillance and care are required from a young age[362].

Mixed species

This section presents entries in the DISARM database concerning prudent AMU in more than one species of farm animal.

In 2010, the Dutch government required the livestock sectors to reduce veterinary AB use by 50% in 2013 and by 70% in 2015 compared to 2009. A study explored differences in attitudes of veterinarians towards AB reduction in farm animals. Vets across species have similar attitudes towards the Dutch policy to reduce veterinary AB consumption by 50%. Less experienced veterinarians and those working with **ruminants** reported more difficulties such as resisting farmers' or colleagues' demands for ABs compared with veterinarians working with **pigs, poultry** or **veal calves**. More experienced vets were less concerned about the vet's role in AMR, and they valued the right to prescribe and sell ABs more. Main perceived reasons for non-compliance with veterinary advice by farmers were related to finances and time restrictions, although veterinarians specialized in pigs, poultry or veal calves highlighted that also conflicting advice from other advisors may be a cause of non-compliance. Less experienced veterinarians might require support to resist pressure from farmers and colleagues, and experienced veterinarians could be educated about risks associated with overuse of AB. Alternative approaches should be identified for veterinarians to preserve a decent income from giving advice and to prevent contradictory advice being a barrier to implement veterinary advices and improve animal health[55].

The FAO (Food and Agriculture organization of the United Nations) published a manual which promotes the prevention of infections and prudent AMU in **pigs** and **poultry**. These livestock sectors generally have the highest AB use. The manual complements national governance and regulatory measures. Additionally, it aims for a prudent and medically efficient AB use without loss in productivity, and it is especially targeted to farmers with commercialized medium- or large-scale production, veterinarians and other animal health personnel in non-EU Eastern European and Balkan countries, the Caucasus and Central Asia. The principles and practices described in the manual are universally applicable. Several highlights include gradually stopping the use of growth promotors and preventive AMU, minimising the use of critical important antimicrobials (CIAs) for human medicine in animals, limiting AMU without prescription, moving toward treating individual animals (avoiding group treatments) and proper disposal of used and expired ABs[102].

Digital training courses are available for employees working in **dairy** farming (in Danish) and **pig** farming (in Danish, English, Ukrainian and Russian)[18].

5.11. Other

No strategies or innovations were placed in this category in the DISARM database, which leads us to believe that the structure of the collection protocol is suitable to include most, if not all, material available on the subjects of AMU and AMR in the main livestock production systems.

6. Conclusion

Reviewing the material collected in the DISARM project showed that a huge variety of publications is present. The top three categories regarding number of entries are antimicrobial use (AMU) reduction strategies (107 records), prudent use (71), pathogen management (61), biosecurity (55), and feed and gut health (46). All categories (except 'other') had entries and most entries are research papers and reports. Pigs and dairy are the top species regarding number of entries (130 and 136 respectively), where also a lot of material concerns multiple species (136 records). Beef and sheep/goats are underrepresented in the database (8 and 19 records respectively). Effort has been put in gathering relevant material for all categories and species included in the study. The collection continued throughout the course of the DISARM project and was fed with material not only gathered by the consortium partners, but also by the Community of Practice formed within Work Package 2 of the project. This Community of Practice consisted of 504 members by the end of September 2021 with a goal of achieving 600 by the end of the project, representing a range of fields of expertise and parts of the production chain and animal husbandry. A lot of material, especially farm and industry innovations, can be sourced by that broad community. Vice versa, the community of practice was used to disseminate strategies and innovations to reduce antimicrobial resistance (AMR) and AMU gathered in the state-of-the-art database. The online database is promoted for use so that experiences can be shared and copied further. The database can be found here: (https://disarmproject.eu/search-resources/).

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7. References not in the database

Note: For references in the database, referred to in the text using green superscripts, see https://disarmproject.eu/search-resources/.

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