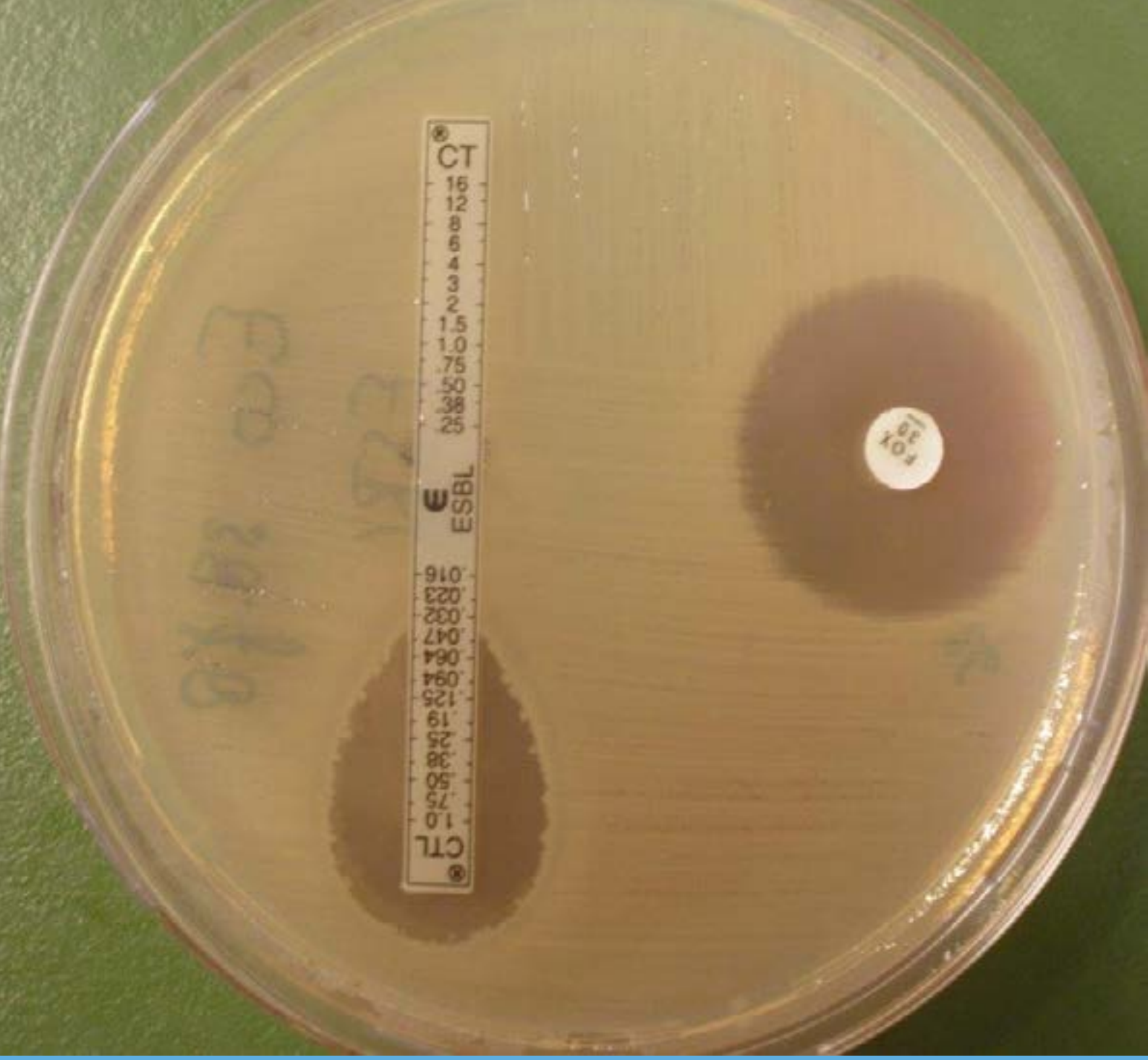

Knowledge Agenda

How to deal with ESBL-producing isolates

C.M. Dierikx, M.H. Bokma-Bakker, R. Bergevoet, D.J. Mevius

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Knowledge Agenda

How to deal with ESBL producing bacteria in the food-chain and the environment

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Samenvatting NL

In deze studie zijn op basis van een literatuurscan en expertview de belangrijkste kennishiaten geïdentificeerd met betrekking tot voorkomen, verspreiding en mogelijke controle van ESBL-producerende bacteriën in de voedselketen en het milieu. De belangrijkste kennisvragen kunnen worden gerelateerd aan vijf thema's: 1) een verdere reductie van het gebruik van antibiotica; 2) het realiseren van een duurzame en gezonde veestapel; 3) het vóórkomen en de spreiding van ESBL's en antimicrobiële residuen; 4) het effectief ontwerpen van interventies, waarbij aandacht is voor de socio-economische omgeving waarin de belanghebbenden functioneren; en 5) als op zich staand thema de rol van gezelschapsdieren bij het ontstaan en de verspreiding van ESBL's naar mensen.

Het recente ECDC/EFSA/EMA first joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals (JIACRA) (http://www.ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-JIACRA-report.pdf) concludeerde op EU-niveau dat: 'er geen relaties bestaan tussen gebruik van 3e - en 4e generatie cefalosporines in voedselproducerende dieren en het vóórkomen van resistentie tegen deze antibiotica in geselecteerde bacteriën van de mens'. Dit in duidelijk contrast met de bevindingen uit Nederland welke gedetailleerd zijn beschreven in de 'Kennis-Agenda'. In dit rapport wordt een gedeeltelijke bijdrage van ESBL's uit niet-humane bronnen aan infecties bij de mens beschreven. Daarom kunnen dierlijke bronnen, via dierlijke producten of andere routes als een potentieel gevaar voor de volksgezondheid beschouwd worden.

Summary UK

This study contains based on a literature scan and expert view the most important knowledge gaps to be addressed regarding occurrence, spread and possible control of ESBL-producing organisms in the food-chain and the environment. The main knowledge gaps can be linked to five themes: 1) a further reduction of antibiotic use; 2) realizing sustainable and healthy livestock; 3) prevention of the occurrence and spread of ESBLs and antimicrobial residues; 4) designing of effective interventions taking into account the socio-economic environment of the stakeholders; and 5) the role of companion animals in the occurrence and spread of ESBLs to humans.

The recent ECDC/EFSA/EMA first joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals (JIACRA) (http://www.ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-JIACRA-report.pdf) concluded that, at the EU level 'there were no associations between consumption of 3rd - and 4th-generation cephalosporins in food-producing animals and occurrence of resistance to this sub-class in selected bacteria from humans'. This is in clear contrast with the findings from the Netherlands described in considerable detail in the 'Knowledge Agenda', which strongly suggest a partial attribution from non-human sources of antibiotic-resistant bacteria and more specifically ESBLs to human infections. Therefore animals, animal products or other sources of contamination can be considered a potential danger to public health.

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Knowledge Agenda

Introduction

The Government has concerns about the occurrence of antimicrobial resistant strains, especially ESBL[1]-producing bacteria in the food-production chain. However there is still insufficient knowledge about ESBLs for an appropriate assessment of the human health risks associated with their occurrence in animals and foods and it is unclear in what areas the knowledge is still insufficient. This knowledge agenda has focussed on relevant information necessary to answer the questions below:

1. To what extent are antibiotic resistant bacteria and more specifically ESBLs in animals as carriers, animal products or other transmission routes a danger to public health?
2. What is the probability that new variants like carbapenemase-producing organisms in animals will emerge and what measures can be taken to prevent this from happening?
3. Which factors have determined the rapid rise in antibiotic resistant bacteria and more specifically ESBLs in animals, and what are the possibilities for control? What are the major knowledge gaps and how can they be addressed?
4. To what extent can the situation in the Netherlands about ESBLs in animals be compared to other EU Member States and countries from which animals or animal products are imported? What is the contribution to the Dutch problems?
5. What management and policy measures may be taken to control antibiotic resistance and more specifically ESBLs in animals? What are the effects of these measures on the risk to public health?

Materials and methods

To answer the five questions a review was conducted of the current literature on ESBL-producing organisms in the food production chain in which expert opinions are included. This literature scan has focussed on existing knowledge concerning how and where ESBL-producing bacteria occur and spread across food chains, how resistant bacteria spread to the environment, what possible preventive measures are known and what knowledge gaps can be defined.

The scope of the report is to identify the main knowledge gaps on the drivers for and the occurrence of ESBLs in livestock (and other animals) and food-products thereof, the environment and the public health risks related to ESBLs. This is based on a description of the current knowledge, which is presented in the appendixes. Based on this description, the abovementioned questions are answered below and the main knowledge gaps identified.

Results – answers to the five questions

Question 1.To what extent are antibiotic resistant bacteria and more specifically ESBLs in animals, animal products or other sources of contamination a danger to public health?

The majority of the types of ESBL genes, plasmids and the bacteria found in health care are different from those found in food-producing animals. This indicates that ESBL-epidemiology in health care and in food producing animals are not directly linked. Two major domains seem to exist: one health care based and one in the food chain. Direct contact of people with animals carrying ESBL-producing bacteria increases the chance of colonization of humans. Possible sources of plasmid mediated ESBL genes for humans are e.g. food, but also the environment. A minority of the genes and plasmids that occur in ESBL-producing isolates from humans in health care are genetically associated with genes and plasmids from poultry and poultry products. For other food animal species there is only an association found on farm between isolates of animals and farmer, but overall, the epidemiological evidence available is limited. However, the distribution pattern of ESBLs in different reservoirs as described above may be specific for the Netherlands, but does not necessarily reflect the pattern elsewhere. Moreover, there are some indications that CTX-M-1, the most abundant ESBL type reported in animals so far, circulates in humans at significant rates as well. This may be more the case within the open rather than the hospitalized population. Unfortunately, most molecular data in humans originate from hospitals, and this

[1] For readability of the document, the term 'ESBL' is used for both ESBL and plasmid-mediated AmpC producers. Both groups confer resistance to 3rd generation cephalosporins, however ESBL-producers are sensitive to combinations with clavulanic acid and cephamycins, and resistant to 4th generation cephalosporins while AmpC producers are resistant to combinations of beta-lactam antibiotics with clavulanic acid and cephamycins (e.g. cefoxitin), and sensitive to 4th generation cephalosporins.

may be considered as a knowledge gap. Currently quantitative information on prevalence and characteristics of ESBL-producing bacteria, the genes and plasmids in the open human populations in the Netherlands are under investigation. It is known though that colonized persons can stay positive with the same strain for many months and even years (depending on the strain and gene characteristics). It is expected that the range of a dose that leads to colonization or infection will be very broad and will depend of many factors. "Infection"(=getting colonized) with ESBL-producing bacteria does not immediately lead to clinical cases, but usually persons are first colonized for some time and this sometimes results in illness later. No large food-related outbreaks have been reported.

Knowledge gaps

1. An important lack in knowledge is the absence of information on dose effect relations for ESBLs (and carbapenemases). In other words, how many ESBL bacteria (independent or dependent of which source (animal versus human)) are necessary to colonize the human gut or result in transfer of the ESBL-genes to human bacteria and to estimate the effect of reduced exposure of humans on colonization? The answer to this question is essential to understand the extent of reduction needed in sources of ESBL bacteria and which measures to be applied in e.g. the food chain to successfully intervene to prevent colonization of the human gut with ESBL (and/or carbapenemase) producers. Commensal *E. coli* harbouring ESBLs is considered a more important source for transfer to humans than *Salmonella*.
2. There is also no information about which dose of ESBL- (or carbapenemase-) producers will ultimately lead to an infection in humans.
3. Good knowledge on the prevalence of the different types of ESBLs in the healthy human population is lacking. This knowledge would help in understanding potential transmission between humans and animals. Current human data is predominantly coming from patients in hospital environments.

Question 2. What is the probability that new variants like carbapenemase-producing organisms in animals will emerge and what measures can be taken to prevent this from happening?

Until now, carbapenemase producers are mainly found in humans. In the Netherlands Enterobacteriaceae with plasmid mediated carbapenemase genes are incidentally observed in human patients and have never been found in livestock or food thereof. Human cases in the Netherlands are currently only associated with human introductions and not to consumption of food. This may change when Enterobacteriaceae with plasmid mediated carbapenemases are introduced in food-producing animals. The circumstances in these production chains facilitate a further spread into the food chain and the environment. Although specific selective antimicrobials (carbapenems, cephalosporins) are not or no longer used in food-producing animals in the Netherlands, co-selection by use of other antimicrobial classes can potentially support the spread of these resistant bacteria. Multi drug resistant organisms like carbapenemase producing Enterobacteriaceae may be introduced in humans through contaminated food (fish and shrimps, vegetables, herbs).

Because of the close interaction between pets and their owners and the use of modern broad-spectrum antimicrobials in companion animal health care (including horses), a possible introduction of carbapenemase producers in companion animals through the owners and subsequent spread due to antibiotic use is considered more likely than in food-producing animals.

Knowledge gaps

1. The risk of introduction of carbapenemase producing Enterobacteriaceae in livestock production or companion animals from human carriers or human waste is unknown.
2. There is a need for a more detailed discussion on the risks related to pets. What will be the threats related to pets? Will they be a source for transmission between family members or a source for livestock? What is the contribution of companion animal clinics to the spread?

To prevent the emergence of carbapenemase-producers, we propose the following recommendations:

1. Constant monitoring of carbapenemase producers in livestock and companion animals is very important to be able to act adequately when they are detected, ideally before they are widely spread. Given the low estimated prevalence selective methods with highest sensitivity should be used.
2. Monitoring of meat products is of second priority and should preferably be done on a risk basis and more focused at fish and shrimps, vegetables and herbs from high risk countries.
3. Full enforcement of the reduction policy of cephalosporin use in companion animals.
4. Development of a contingency plan for control of carbapenemase producing bacteria once they have been detected in the food chain or companion animals.

Question 3. Which factors have determined the rapid rise in antibiotic resistant bacteria and more specifically ESBLs in animals, and what are the possibilities to control this? What are the major knowledge gaps and how can they be dealt with?

Antibiotic use, especially beta-lactam and cephalosporin use, and the way our intensive livestock production is organized has facilitated the rapid selection and spread of ESBLs in animals. Also residues of antimicrobials in the farm environment may play a role in selection for antimicrobial resistant bacteria including ESBLs. In addition to these environmental influences, the molecular characteristics of the resistance determinants in evolutionary adapted organisms and plasmids have supported the rapid spread of resistant bacteria. Reduction of antimicrobial use in Dutch livestock production has already led to a decrease in cefotaxime resistant commensal *E. coli* bacteria. However, data from other countries where ESBL bacteria occurred even without the use of antibiotics show that other factors like import of contaminated animals and spread through production chains are also important. Reduction of antibiotic use is necessary to reduce selection pressure, but will probably not lead to complete disappearance of ESBL bacteria in animal production.

Existing data suggest that the causes of the rapid rise in ESBLs in animals differ depending of the animal sector and by country for a definite animal sector.

Knowledge gaps

1. It is unknown what the effect is of residue concentrations of antimicrobials on the selection and evolution of antimicrobial resistance. Examples are residues in milk from treated cows or residues of antimicrobials excreted in the farm environment through urine or faeces. At this moment it is unknown to what extent these residues are present, how long they are present, and how these residues will influence the occurrence of antimicrobial resistant bacteria in the gut and litter/slurry and whether this is important for the spread of antimicrobial resistant bacteria into the farm environment and the food chain.
2. At the moment there is still not enough information on which reduction of the use of antimicrobials in livestock will lead to the occurrence of resistance found in bacteria derived from livestock (including ESBLs). It is expected that the reduction curve of resistance will be a non-linear process and that this will likely not exactly mirror the reduction of the usages. A levelling off at certain stage can be expected.
3. It is unknown which other factors than antibiotic use contribute to the occurrence of ESBLs.
4. The understanding of the spread of resistant bacteria or resistance determinants into the environment and whether this has an impact on their reintroduction into the farms, on vegetable products or direct exposure of humans is unknown.

These knowledge gaps can be dealt with by:

1. Research that investigates no-effect levels of residues.
2. Taking measures to prevent the presence of antimicrobial residues in milk and farm environment above no-effect levels.
3. Risk factor analyses related to ESBL presence at farms.
4. Development of sustainable and healthy livestock production. As a result less antimicrobials will be used. Examples of farming practices in different livestock sectors that currently successfully produce animals with minimal or no antibiotic use should be used to promote such farming types.

Question 4. To what extent can the situation in the Netherlands about ESBLs in animals be compared to other EU Member States and countries from which animals or animal products are imported? What is the contribution to the Dutch problems?

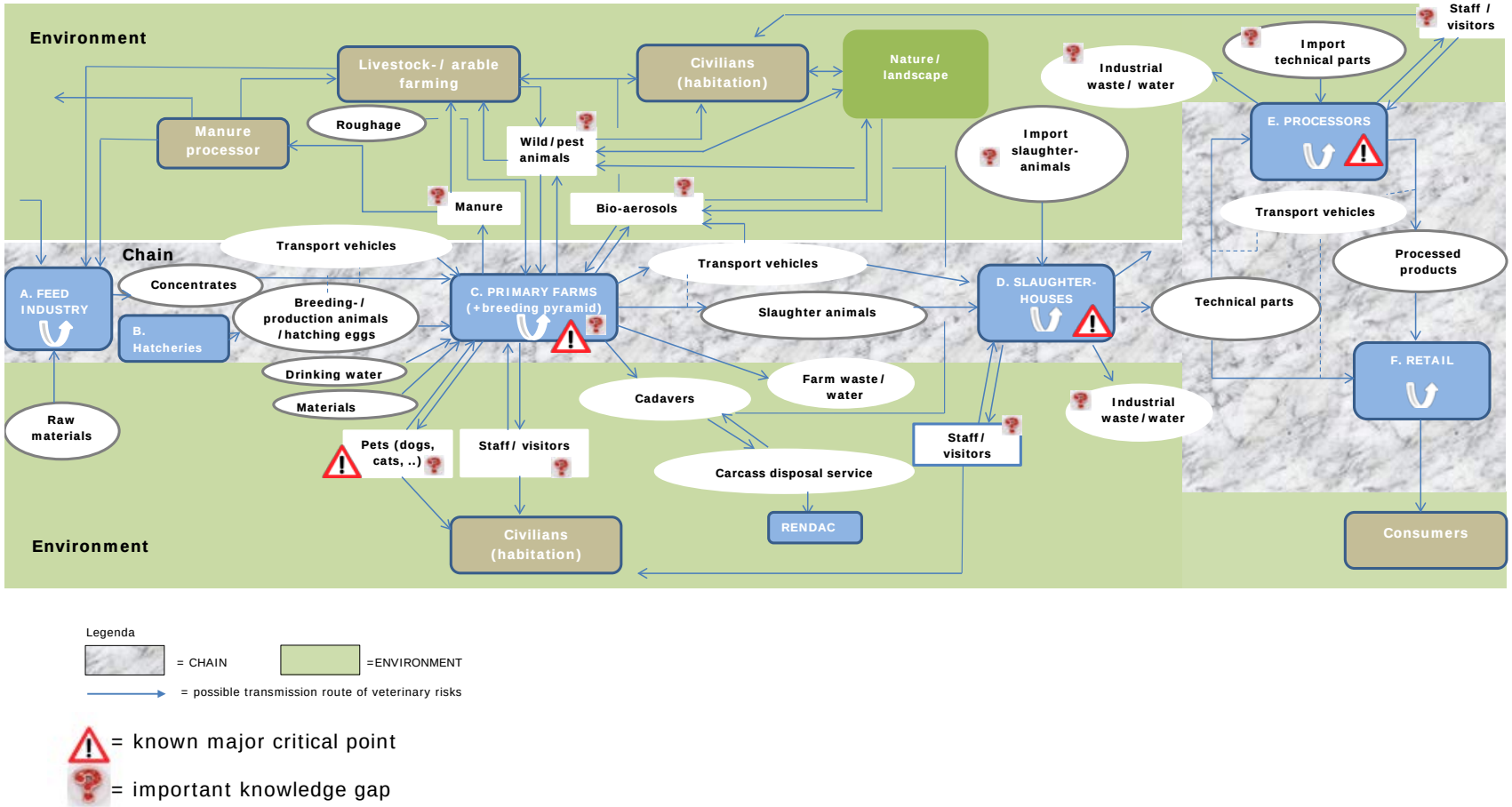
Except for a few countries which are known to have lower ESBL levels in animals (Scandinavian countries) the situation in EU countries is comparable to the Netherlands (Germany, Belgium) or even worse (Spain, Greece). It is also known that poultry meat from Brazil is regularly found to be contaminated with ESBL producing strains. The import of animals and animal products is expected to increase in the near future.

Human carriers can introduce ESBLs in animals and livestock. E.g. through hospitals and travel.

Knowledge gaps

1. Quantitative information on ESBLs in food and live animals derived from other countries is lacking. However, there are sufficient indications at the moment to consider policy measures to be implemented in order to distinguish imported versus domestic animals and animal food products at various stages of the food chain.

2. In the past in the monitoring program on ESBL producing bacteria on meat from Dutch supermarkets, done by the NVWA, the country of origin was not always known and therefore it is not known how much import from other countries existed. If ESBL levels in Dutch animal production system will improve, the contribution of import (from animals or meat) to Dutch ESBL problems will be more significant.



Legenda

= CHAIN =ENVIRONMENT

= possible transmission route of veterinary risks

= known major critical point

= important knowledge gap

Figure 1 *Schematic representation of animal food production chains and possible transmission routes of veterinary risks in the chain and to plant environments.*

Question 5. What management and policy measures may be taken to control antibiotic resistance and more specifically ESBLs in animals? What are the effects of these measures on the risk to public health?

Figure 1 provides a schematic representation of animal food production chains and possible transmission routes of veterinary risks to the food chain and to plant environments. The precise attribution of each animal source to the occurrence of ESBL-producing bacteria in the food chain is unknown and needs to be based on attribution modelling projects. The ongoing public-private research project "ESBL attribution" will provide more insights in this matter, including the contribution of animal sources and the food-chain to human carriership. The available current data are insufficient to identify risk factor and in general miss longitudinal information.

Based on current knowledge we identify the following major critical control moments in the food chain (see figure 1 ⚠):

1. *Pets and primary farms including breeding pyramids.*
   Control measures should focus on strict limitation of use of antibiotics, especially cephalosporin use, and on strict implementation of on-farm external and internal biosecurity measures. Special attention must be given to prevention of transmission by contaminated animals through breeding pyramids (vertical transmission) or between production farms (horizontal transmission). Moreover, in livestock production active monitoring should be implemented to identify the introduction of such bacteria into the breeding level.
   Monitoring of ESBL-bacteria (and carbapenemase-producers) at primary farms and logistic slaughtering of positive herds, would facilitate the control of these bacteria.
   Although not included in figure 1, the use of those antimicrobials considered critically important in humans should be minimized in pets. Drugs currently not licensed for veterinary medicine should not be used at all (no off label use).
   Measures to monitor and possibly control off-label usage of antibiotics should be considered and implemented.

2. *Slaughterhouses and meat processors.*
   Control measures should focus on elimination or reduction of (re-)contamination of carcasses with coliform bacteria due to contaminated meat, slaughter equipment and human handling during meat processing. Attention should be paid to minimize the risk of introduction of bacteria including ESBLs via imports of slaughter animals or carcasses/meat from countries with an unknown or unfavourable contamination -status, e.g. by a selective purchasing policy or by logistic processing of imported animals (separated from domestic animals). Decontamination of carcasses or meat as end-of-pipe solution at slaughterhouses or meat processors is not seen as a plausible step in the control of ESBLs or other bacteria in food chains, because preferably the appearance and spread within and between the chain stages should be targeted to reduce contamination of end products and reduce the risk of transmission of bacteria to the slaughter plant's environments. However, if in the future carbapenemase-producing bacteria might become introduced into animal production chains, then these most likely will have been introduced by human sources. In the case of carcass or meat contamination with bacteria producing carbapenemases, a decontamination step as end-of-pipe solution might then be considered. Therefore development of novel, safe and acceptable techniques to reduce and control infective loads at carcasses and/or meat is a priority.
   To prevent the introduction of carbapenemase producing isolates by humans into the food production chains, regular bacteriological examinations of staff, e.g. after a journey to high prevalence countries, after a stay in the hospital and after a period of diarrhoea could be considered.

3. *Socioeconomic focus points (not in figure 1).*
   To successfully implement interventions aiming at reduction or elimination of ESBLs in animal food chains, attention must be given to those mechanisms that effectively change farmer's behaviour. Cost-effectiveness on farm level, practical feasibility, relevance at sector level, societal impact, absence of undesirable side effects and the behavioural characteristics of the person/farmer all determine the potential success of implementation of interventions. Incentive mechanisms like bonus or penalties intelligently used will facilitate a successful implementation. Stimulation of awareness

through ‘tailor made’ information, education and training will also help to strengthen entrepreneurship, knowledge and skills of farmers and personnel and facilitate required behaviour. As explained in the report, the control of ESBL spread does not only rely on strict limitations of usage and monitoring of resistance. Socioeconomics is highly important and covers a wide range of domains which mostly question the existing livestock production systems in developed countries. As an example, no technologies necessarily address the issue of decontaminating antibiotics or antibiotic-resistant bacteria (such as ESBLs) from hospital or farm effluents. Therefore, dealing with ESBLs in the future will require a global insight into the role of several sectors and interactions between them, with particular attention to a novel management of environmental issues.

Furthermore, we identify a number of major knowledge gaps that preferably should be addressed to support balanced policy-making (see figure 1).These main knowledge gaps identified on the topic of ESBL-producing bacteria can be linked to five themes:

1. Reduction of antibiotic use.
Antibiotic use is considered to be the major factor involved in selection of ESBL-producing organisms. It is unknown if the current reduction of antibiotic use in livestock in the Netherlands is sufficient to control the occurrence of ESBLs in animals and the food chain. Questions that remain are:

- What is the effect of further reduction in use of selective antibiotics (all beta-lactams) for livestock production and for the occurrence of ESBLs? (*primary farms)*?
- The question “what is the effect of further reduction in use of antibiotics” should be: “can we continue to decrease antibiotic use and to what level?” We know little about other than that this topic is a research agenda in itself. To do so, differences in antibiotic use between farms and prescription pattern of veterinarians should be related to critical success factors. (*primary farms + breeding pyramid*)
- What is an acceptable level of ESBLs in animals and products thereof that poses a reduced burden to Public Health? (*primary farms + processed products*/*retail*)
- It is not known what the effect of the 60% reduction of the use of antimicrobials in animals in NL is on antimicrobial resistance in humans in NL. This information would give an impression of the relative importance of the contribution of Dutch livestock to the total “disease burden” in humans.
- Which other factors than antibiotic use favour the occurrence of ESBLs. This may include factors that have a selective property and factors that are involved with spread of ESBLs in farms, and in animal production chains? (*primary farms + breeding pyramid*)
- What is an acceptable level and kind of antibiotic use on farms that minimizes the risks for selection of antimicrobial resistant organisms of Public Health concern? (*primary farms + breeding pyramid*)

2. Sustainable and Healthy Livestock
It can be concluded that the internationally organized animal production chains have facilitated the international spread of multi-drug resistant organisms like ESBL-producers. To control this spread and prevent the international emergence of future ESBL-like resistance traits the transport of live animals carrying these organisms need to be taken into account. Question of relevance are:

- Is it possible to adapt livestock production systems to diminish transmission of ESBLs between different stages in production chains? (*chain)*
- What is the contribution of imports of live animals to the ESBL-situation in Dutch animal production chains? How can (re-)introduction of ESBLs by animal imports in the future be prevented or reduced? (*imports)*
- What is the role of staff/personnel and visitors in transmission of ESBLs to farms and slaughtering/processing plants and from farms and plants to social networks? (*staff/visitors)*

3. Occurrence and spread of ESBLs and antimicrobial residues
Recent data indicate that a large proportion of Dutch livestock (pigs, calves) have residues of antibiotics that are used for treatment of these animals in their faeces in low concentrations. Although in general no residues are found of drugs that specifically select for ESBLs (e.g. cephalosporins), co-selection may occur through residues of other antimicrobial classes. Because this manure (which is often also ESBL-positive) is used to fertilize the land on a large scale there is a list of questions that need to be answered:

- What is the effect of these residues on the occurrence and characteristics of antimicrobial resistant organisms in these animals?(*manure + primary farms*)

- What is the effect of antibiotic residues in the farm environment on the selection and spread of antimicrobial resistant strains within and between farms? (*primary farms)*
- What is the contribution of ESBLs and/or antibiotic residues through manure on the environmental load (soil, surface water, groundwater, arable crops)? What is its relevance for human health? How can treatment of manure influence ESBLs and/or antibiotic residues loads? (*manure*)
- What is the effect of these residues on the ecology (microbiota) in the farm environment, soil and surface water?
- What is the contribution of transmission of ESBLs and/or antibiotic residues through manure to the environmental load? What is its relevance to human health? (*manure*)
- What is the contribution of ESBLs in wastewater from slaughterhouses/meat processors on the environmental load? What is its relevance to human health? (*wastewater*)
- What is the ESBL-contamination rate of rodents, insects, and wild birds in animal production areas? What is the relevance of these animals for introduction of ESBLs to farms and from farms to the environment? What is the relevance for human health? (*pest animals*)

4. Socioeconomic focus points (not in figure 1)

The main driver of the occurrence of antimicrobial resistance is the quantity and the kind of antibiotic use. In livestock production antibiotic use was predominantly influenced by economic pressure and welfare. Before the successful implementation of antibiotic measures the economy was considered to be the major driver for systematic preventive use of antibiotics. However, the rapid reduction of antibiotic use since 2008 of almost 60% has shown that the vast majority of this use was not necessary for a farmer to produce animals cost-effectively. Moreover, the awareness of farmers of their responsibility to produce animals and products that are safe for public health has increased. Therefore, the following questions remain relevant about the behaviour of farmers and how to influence this behaviour.

- How can conceptual socioeconomic knowledge be adequately used in the design of effective intervention strategies targeting ESBLs?
- What should an integrated approach of multiple intervention mechanisms targeting ESBLs look like in order to reach a broad selection of the farmer population?
    - What are important (socioeconomic) determinants explaining the differences between farmers and vets with high and low usage of antibiotics and how can these determinants be utilized to achieve further reduction?
- What are the exact mechanisms motivating farmers to implement behaviour aiming at preserving public goods (and not perceiving individual benefits) and how should these insights lead to effective intervention strategies?

5. Companion animals

The risk for public health is perceived to be predominantly attributed to livestock that are a source of ESBLs through their faeces, contaminated food-products thereof and environmental pollution through the use of manure. The role of companion animals (e.g. dogs, cats) seems to be somewhat underestimated. Large proportions of healthy dogs carry ESBLs in their faeces. Given their role in families as full family members, a transmission of ESBL and other transferable genes between the family members and these animals is likely to occur.

Given the development of hospital like specialized animal clinics, a development towards infections confined to these clinics is likely and already documented for antimicrobial resistant animal pathogens and resistant pathogens with a zoonotic potential. A “Maasstad hospital” like prolonged outbreak with OXA-48 producing Enterobacteriaceae has recently occurred in an animal clinic in Germany. Because of the intense contacts between owners and their companion animals, the use of third-generation antibiotics in diseased animals and insufficient or lacking infection control policies, such an outbreak may also occur in Dutch veterinary clinics.

Questions of relevance are:

- What is the risk of companion animals as source of ESBL (and carbapenemase)-producers for their owners and for public health? (*pets*)
- Is the current control of the use of third-generation antibiotics in companion animals sufficient to prevent selection of antimicrobial resistant organisms of public health concern? (*pets*)
- What infection control policies exist for antimicrobial resistant organisms in companion animal clinics that pose a risk for animal and public health? (*pets*)

Knowledge Agenda expert opinions

Ten (inter)national experts were contacted to give their opinion on the Knowledge Agenda. All experts were asked to answer two questions:
1. Do you think that the most important knowledge gaps are mentioned in part 1 (page 4-11)? Did we miss any important knowledge gaps? If so, please explain which knowledge gaps in your opinion should be added.
2. Do you think the policy measures (Part 1, Question 5, page 9) described to control antimicrobial resistance and more specifically ESBL bacteria in animals are able to limit or control the problem? Please, give your comments and/or additions on the measures described.

Included in the list below are the opinions of the following seven experts (in alphabetical order) that responded to the request:
Prof. dr. Jeroen Dewulf, associated professor in Veterinary Epidemiology at the Faculty of Veterinary Medicine of the Ghent University, Belgium
Dr. Engeline van Duijkeren, Veterinary microbiologist at the Laboratory for Zoonoses and Environmental Microbiology at the National Institute for Public Health and the Environment, the Netherlands
Dr. Annemarie Kaesböhrer, Head of the National Reference laboratory for antimicrobial resistance in animals, food and the environment at the Bundesinstitut für Risikobewertung, Berlin, Germany
Dr. Jean-Yves Madec, Research Director at the French Agency for Food, Environmental and Health Safety (ANSES) in Lyon, France
Prof. dr. John Threlfall, member of EFSA panel on Biological hazards (BIOHAZ), EMA antibiotics working party (AWP), EFSA/Public Health England, UK
Ing. Jan Workamp, Sector manager Poultry, Animal Health Service, Deventer, NL
Prof. dr. Jaap Wagenaar, Professor in Veterinary Microbiology, Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, NL
Prof. dr. ir. Dick Heederik, professor in Environmental Epidemiology, Institute for Risk Assessment Sciences, Faculty of Veterinary Medicine, Utrecht University, NL

Knowledge gaps (anonymized comments and knowledge gaps based on responses of the experts are listed in random order (each bullet-point refers to the response of a different expert)) and where possible related to the five questions answered in the knowledge Agenda (page 4)):

- Two questions were specifically asked, one on the most important knowledge gaps, the other on the policy measures to control ESBL spread. Before that, I would like to underline the high quality of the review performed and the strong relevance of the propositions expressed. I basically concur with the opinions of the authors and my comments should be read as complementary views only.

Knowledge Agenda Question 1 (page 4).

- I agree that commonalities between ESBL genes, plasmids and clones between animals and humans are weak, with the exception of specific exposures, as mentioned (farmers, pets' owners ...). Nevertheless, there are some indications that CTX-M-1, the most abundant ESBL type reported in animals so far, circulates in humans at significant rates as well. This may be more the case within the open rather than the hospitalized population. Unfortunately, most molecular data in humans originate from hospitals, and this may be considered as a knowledge gap.

  The point on dose effect relations for ESBL is essential. My view is that the animal contribution to the ESBL burden in humans has been often exaggerated in the past thanks to frequent references to ESBLs in foodborne pathogens. Indeed, ESBL transfer from animals to humans through Salmonella is a real issue but also a very rare event. A recent paper (JAC 2014) reported 0.49% of human Salmonella infections associated with ESBL/AmpC production. On the contrary, colonisation of food products with ESBL *E. coli* isolates is being increasingly reported, even though their presence on foodstuff surfaces does not necessarily attest of an animal source. This highlights to what extent the commensal flora (*E. coli*) is probably a more important vector of ESBL transfer (if any) between

animals and humans, and dose/effect approaches are obviously lacking to quantify this risk, which refers principally to colonization.

- I agree to some extent with your knowledge gaps, although I do not agree with your statement on the distribution patterns of ESBL-genes. The situation described seems to be true only for the Netherlands.
  Another major knowledge gap is - in my mind - the understanding, which factors trigger the transfer of the ESBL-gene carrying plasmids to other bacteria, e.g. in the human intestinal tract. At least for our situation, the problem is not just the homology of ESBL-genes in the different populations, but that the genes are found on different plasmids and on different core genomes of E.coli.

- Dose effect relation for ESBL: This is difficult to study as ESBL-producing bacteria colonize the gut and there is currently no method to decolonize carriers of these bacteria. Therefore, studies with health volunteers are difficult to perform for ethical reasons. From the literature and a yet unpublished study, it is known that colonized persons can stay positive with the same strain for many months and even years. In addition, *E. coli* is not a pathogen as such, but causes opportunistic infections. These infections depend on the virulence of the bacterium and the immunity of the host. Therefore, the dose effect will probably differ significantly between persons and also between different E.coli strains. In addition, the dose effect is not limited to clonal transfer of ESBL-carrying bacteria, but also to horizontal transfer of ESBL-genes, which is difficult to measure. It is expected that the range of a dose that leads to colonization or infection will be very broad and will depend of many factors. “Infection”(=getting colonized) with ESBL-producing bacteria does not immediately lead to clinical cases, but usually persons are first colonized for some time and this sometimes results in illness later. No large food-related outbreaks have been reported.

- I would enlarge answer 1 for Q1 also to direct contact. As it is written now it focusses predominantly on the food chain which may give the impression that either direct contact with farm or companion animals is not important or the dose-response curve is known in such a situation.

  I would add to the knowledge gaps here the absence of good knowledge on the prevalence of the different types of ESBLs in the healthy human population. This knowledge would help in understanding potential transmission between humans and animals. Current human data is predominantly coming from patients in hospital environments.

- I miss a reference to changes in exposure of humans. (e.g. ''to estimate the effect of reduced exposure of humans on colonization'')

  The relation between dose of ESBLs/or CPs with infection should be translated to relation between carriership and infection.

Knowledge Agenda question 2 (page 5):

- The size of the risk can’t be calculated but we should be aware that the probability of introducing carbapenemases-producing strains into our livestock populations is far away from zero, saying it is quite realistic. There is another knowledge gap which should be highlighted. What about the risks vegetable products may pose? They can get contaminated during production in our own country (e.g. by water) but also we could import contaminated products e.g. herbs and spices (as shown in the Netherlands).

  There is a need for a more detailed discussion on the risks related to pets. What will be the threats related to pets? Do we expect that they transfer bacteria from one household member to another (which might also happen by direct contact between humans) or do we expect that pets are transferring these resistance genes / resistant bacteria to our livestock population? I think the impact of a problem present in the livestock population might be much higher compared to that of pets. For risks related to pets, the humans can act much more directly, which is not very realistic for risks related to foods.

Another issue: Please include horses in your list as vets tend to use there also carbapenems etc.

What about the role of migratory birds? They could transfer the genes from (human) environments into our livestock populations. This could be quite important if we 'improve' livestock conditions toward outdoor farming.

- I agree with the little knowledge on the risk of introducing carbapenemase-producers in the animal population. This has already happened and will probably happen again. As mentioned by the authors, an issue would be that such an introduction will be followed by an amplification and uncontrollable situation through successful clones and/or massive co-selection of plasmids by the use of other antimicrobials widely used in veterinary medicine. Considering the high diversity of genetic combinations, this risk should probably be considered differently depending of the carbapenemase types. For instance, OXA-48 emerged in pets in Europe and might be found incidentally in livestock in the future. However, OXA-48 is mostly found on the same plasmid type worldwide, which does not harbour many other resistance genes. The situation may be drastically different with other carbapenemases (such as NDM-1), which can be located on a variety of multi-drug resistant plasmids.

- I would be a bit more prudent in the last sentence: "Because of the close interaction between pets and their owners and the use of modern broad-spectrum antimicrobials in companion animal health care, a possible introduction of carbapenemase producers in companion animals is more likely than in food-producing animals". I agree on the fact that companion animals pose a real threat. Moreover some carbapenems are registered for companion animal (topical) use, at least in Belgium. But I'm not sure if the food-producing animals are safe? Given the huge level of trade between animals throughout Europe, but especially with the close neighbours, it could very well be that in countries where for instance 3° gen cephalosporins are still used the CPE's are selected and that these are subsequently exported through trade. Once they get into an intensive production system they may spread very easily as you have correctly pointed out. It has been observed in Sweden for example that there has emerged a high prevalence of ESBL carrying *E. coli* (without use of cephalosporins) which is believed to be imported.

Knowledge Agenda question 3 (page 6):

- Another knowledge gap is the understanding of the spread of resistant bacteria or resistance determinants into the environment and whether this has an impact on their reintroduction into the farms, on vegetable products or direct exposure of humans.

  A risk factor analysis on the farm level has been tried for broiler farms, but due to the farm prevalence of app. 100%, this was impossible. For risk factor analysis, a significant number of positive and negative farms are needed.

  Interventions regarding the environment are generally difficult to implement. Contamination of the environment will decrease when the prevalence in animals and humans decrease. Also, I personally believe that exposure through the environment is less important.

- I have no major comment on the main factors determining the rise of ESBLs in animals as the authors have widely covered this topic. I agree that the magnitude of the environmental component (including residues) of the ESBL cycle is poorly known. I would add that the causes of the rapid rise in ESBLs in animals surely differ depending of the animal sector. Things may also vary depending on countries for a definite animal sector. Therefore, beyond general guidelines, efficient policy measures should closely stick to local practices in farms (here, Dutch farms), which may differ from other successful practices in other countries.

  In line with the need of limiting the impact of residue concentrations on the selection - or co-selection - of resistant bacteria such as ESBL producers, research on existing antibiotics that would be chemically modified, such as to get minimum persistence in the environment or be excreted

through the urinary tract (compared to gut) would probably help. Such an expected positive impact on the control of ESBL spread is however still speculative for now.

- In the first sentence of the answer I would also refer to general Beta-Lactam use. Ones ESBLs are introduced in the population they are also promoted through Beta-Lactam use other than cephalosporins.

  It is expected that the reduction curve of resistance will be a non-linear process and that this will likely not exactly mirror the reduction of the usages. Maybe there will be levelling off at certain stage. Better understanding the relation between antimicrobial usage and resistance is therefore crucial to be able to predict where this is going to.

- The advice for development of sustainable and healthy livestock production seems to be a very general and in this report not well documented statement. This is in conflict with farmers and farming initiatives in different conventional Dutch livestock sectors that manage to produce animals with minimal or no use of antibiotics.

- At this moment it is unknown to what extent these residues are present and how long they are present.

Knowledge Agenda question 4 (page 6):

- The point of importation of food products is major but a clear quantitative picture is globally lacking, and this is obviously a major knowledge gap. However, there are sufficient indications at the moment to consider policy measures to be implemented in order to distinguish imported versus domestic animals and animal food products at various stages of the food chain.

- I would also mention here the risk of introduction of resistance through trade of live animals.

  Figure 1: It is worth adding the risk of introduction from humans to the animals – food production chain. E.g. through hospitals etc.

Knowledge Agenda question 5 (page 9):

Missing policy measures according to the experts:

- To answer this question correctly, more understanding of the epidemiology and attribution of ESBL-bacteria is warranted based on attribution modelling projects. This is an element that needs to be emphasized.

  Further, there is currently a lot of study material on ESBLs, but still little longitudinal information.

  A problem is that the current surveillance is not optimal. It can identify trends, but if more information would be available on the sampled farms or herds, this would result in more optimal analysis of the data.

- As regards livestock production there should be a clear recommendation that active monitoring should be implemented to identify the introduction of such bacteria into the breeding level.

  Furthermore a recommendation is missing which addresses pets. The usage of those antimicrobials considered critically important in humans should be minimized. Drugs currently not licensed for veterinary medicine should not be used at all (no off label use).

  In the slaughterhouse, there should be also worked on the prevention of the introduction of bacteria into the slaughter process, e.g. by contaminated animal surfaces. As regards the slaughter process for poultry there should be a critical assessment of the process and the underlying cleaning and disinfection steps.

As regards the introduction of carbapenemases producing isolates by humans into the food production chains, there might be an important measure to improve and re-implement regular bacteriological examinations of staff, e.g. after a journey to high prevalence countries, after a stay in the hospital and after a period of diarrhoea.

- Maybe attention should be given to some of the recommendations given to the EC in response to their requests for scientific advice on the impact on public health and animal health of the use of antibiotics in animals (the AMEG report, published in December 2014). In addition to the policy measures in the 'Knowledge Agenda' Part 1, Question 5, page 9, in the AMEG report it was stated that:
  'A number of risk management options have already been implemented at the EU/national level. The need for further risk management measures should be based on evidence and on a dedicated risk assessment. Measuring the impact of individual risk management measures is difficult, but efforts should be made to evaluate the effectiveness of such measures by means of agreed criteria. Assessment of the EU-wide impact of new risk management measures requires the development of internationally-agreed systems that are capable of measuring their success or failure through adequate monitoring systems of antimicrobial sales/use and resistance. Such monitoring systems may include:
  - Monitoring by ESVAC of changes in antimicrobial consumption in particular of fluoroquinolones and cephalosporins as a means to measure impact of actions implemented.
  - More precise data by animal species/ livestock production categories in future ESVAC reports, including e.g. the use of DDDA and DCDA.
  - Prescribers should keep records of off label use to be provided at the request of the Authorities.
  - Authorities should be encouraged to collect off label use data.
  - Regular joint analyses of the evolution of antimicrobial resistance and sales/use by the Joint Interagency Antimicrobial Consumption and Resistance Analysis (JIACRA) EU expert group.

The AMEG report proposes that in addition the following activities should be carried out:

- Reduction of overall antimicrobial consumption.
- Promotion of good farming practices and animal husbandry.
- Further research into the off label use of antimicrobials in animals; actions could be derived from the result of research findings.
- Further research into pathways of dissemination of AMR bacteria from animals to food and also into methods for the quantification of the spread of resistance genes from commensals to pathogens in foods and the environment.
- Further research into the extent of metaphylactic use of orally administered AMs and the impact of this practice on the development and persistence of resistance in the gut microflora of the animal.
- Researching methodologies to evaluate the potential economic consequences and impact on both human and animal health and welfare that would result from the introduction of new risk-based measures.

Some, but not all of potential control measures have been stated in Part 1, Question 5, page 9 of the 'Knowledge Agenda'. Measures such as monitoring and possibly controlling off-label usage.
The off-label use of antibiotics is a 'hot topic', and may merit more attention in the document.
It is questionable if residues are an important issue to consider. They have been explored in some depth in the past, and was a subject looked at by the UK DARC group some time ago.

The effects of existing control measures should be properly analysed before any 'new' restrictions on usage are imposed.

- As regards the recommendations, I don't agree that foods should not be monitored. In foods focus should be given to vegetable products and products imported from third countries, e.g. aquaculture products. But we may also remember the EHEC outbreak, there sprouts were a very risky product.

  Another aspect which should be highlighted is that we should have a very close look on the top

breeding level, both for poultry and pigs. Some kind of a mandatory surveillance system should be envisaged.

Monitoring of CPE should not be limited to animals at the slaughterhouse, but should include the whole production pyramid.

- Constant monitoring of CPE in livestock is needed, as mentioned by the authors. I would underline the importance of taking the opportunity of the still favourable situation of a very low prevalence of carbapenemases in animals to monitor the commensal flora through selective media, which was unfortunately too much delayed in my view for ESBL producers at the European level.

  I would also suggest clear policy on the forbidden use of carbapenems in companion animals in Europe and the need for more frequent integrated actions with the human side, such as considering appropriate measures associated with risk factors of introducing carbapenemase producers in the animal population when pets' owners or farmers were treated with carbapenems or are at known risk of carriage of carbapenem-resistant bacteria.

- As perfectly explained in the report, the control of ESBL spread does not only rely on strict limitations of usage and monitoring of resistance. Socioeconomics is highly important and covers a wide range of domains which mostly question the existing livestock production systems in developed countries. As an example, no technologies necessarily address the issue of decontaminating antibiotics or antibiotic-resistant bacteria (such as ESBLs) from hospital or farm effluents. Therefore, dealing with ESBLs in the future will require a global insight into the role of several sectors and interactions between them, with particular attention to a novel management of environmental issues.

- Knowledge on transmission of ESBL-bacteria in production pyramids is currently insufficient to target preventive measures.

  Monitoring of ESBL-bacteria (and carbapenemase-producers) at primary farms and logistic slaughtering of positive herds, would facilitate the control of these bacteria.

- Control measurements should be focussed at the primary phase, at the production farms. Reduction at this level will also affect environmental reduction and reduction in slaughter plants.

Other important knowledge gaps that may be considered are:

- the relative contribution of food-producing animals to the number of clinical cases in human medicine or to the number of colonized persons in the community compared to other routes (travel, human-to-human transmission, human antimicrobial usage etcetera);
- the relative importance of the different routes of exposure of humans, e.g. contact versus food versus environment;
- it is not known what the effect of the 60% reduction of the use of antimicrobials in animals the NL is on antimicrobial resistance in humans in NL. This information gives an impression of the relative importance of the contribution of Dutch livestock to the total "disease burden" in humans;
- transmission dynamics of antimicrobial resistance determinants is a very complex ecosystem; reduction of the exposure by one route might lead to the increase of the importance of other routes (e.g. number of human Salmonella cases due to travel increased after the successful reduction in livestock in Scandinavian countries);
- the effect of co-selection: ESBL or carbapenemase producing bacteria are often MDR and therefore the effect of other classes of antimicrobials other than ESC/beta lactams might be of significant importance;
- the relative contribution of imported products to the total exposure of humans in NL through the food chain;
- the effect of contamination rate versus consumption pattern is unknown. Chicken meat is generally heated before consumption while other food products like filet americain are consumed raw.

- Analysis of co-resistance determinants in plasmids associated with ESBL production.
  The use of antibiotics other than ESBLS but for which determinants for resistance are physically linked to ESBL determinants on plasmids can ‘silently’ promote the spread of ESBL resistance. Perhaps the presence of such determinants needs to be quantified in relation to antimicrobial usage in food-producing animal species.
- International control of the use of carbapenem antibiotics in animals through CODEX.
  Although not officially documented, there is increasing evidence that carbapenem antibiotics have been used in some countries in the Far East in animal production. Such usage should be banned, and imports of food animal products from such countries banned through CODEX regulations.
- The recommendation to control pest animals at farms accounts also to other sources of introduction of ESBL-bacteria such as wild birds. The question “what is the effect of further reduction in use of antibiotics” should be: “can we continue to decrease antibiotic use and to what level?” We know little about other than that this topic is a research agenda in itself. To do so, differences in antibiotic use between farms and prescription pattern of veterinarians should be related to critical success factors.
- The knowledge gap: “What should an integrated approach of multiple intervention mechanisms targeting ESBLs look like in order to reach a broad selection of the farmer population? Seems to be over-focussed at economy and behaviour. Why not analyse differences of farms with high and low usage or similar with vets? This will result in determinants for further reduction in use.
- What is the attribution of each source to the total exposure of humans in NL? This is already under research in the ESBLAT research program, but the data need constant updating in the coming years.

Appendix 1 *Background information (literature scan)*

Table of contents

1. Background on ESBL-producing bacteria

1.1 The origin of antimicrobial resistance

The environment is considered to be the natural reservoir of resistance genes. Soil bacteria, especially bacteria belonging to the family of Actinomycetes are known to produce antibiotic resistance proteins to protect themselves from antibiotics they produce themselves. Possibly antibiotics produced by these bacteria exert selective pressure on other microorganisms in the same habitat as well. The existence of these resistance genes, as well as precursor proteins that originally have alternative biochemical functions but can easily change into resistance proteins in the case of selective pressure, are considered the source of resistance in human and animal bacterial populations (RIVM, 2010).The existence of natural resistance genes is demonstrated by the presence of low frequencies of resistance genes in *E. coli* isolated prior to 1950, before antibiotics were widely used, and the presence of antibiotic resistance in natural, remote environments. The use of antimicrobial drugs in human and animal healthcare, as well as additional applications such as aquaculture, crop protection, animal feed additives and food conservation has resulted in the widespread development of resistance not only in bacteria in humans and animals, but also in the environmental reservoir (RIVM, 2010).

1.2 ESBL-producing bacteria

1.2.1 Increase of ESBL-producing bacteria outside the hospital setting

Monitoring programs on antimicrobial resistance in human pathogens show increasing numbers of bacteria resistant to critically important antimicrobials for human health, like the fluoroquinolones and 3rd and 4th generation cephalosporins. Especially bacteria producing Extended Spectrum Beta-Lactamases (ESBLs), which makes them insensitive for the newer generation cephalosporins, are increasingly found. The development of the newer generations of cephalosporins with their broad (extended) spectrum against both Gram-positive and Gram-negative bacteria has probably been the driving force for the recent emergence of ESBLs. ESBLs have first been described a few years after the third generation of cephalosporins were licensed for therapeutic use in humans. Also in veterinary medicine, the increase in ESBLS shows parallels with the availability of third and fourth generation cephalosporins in the veterinary field (RIVM, 2010). Spread of these resistant bacteria in the Netherlands is minimized by strict hygienic rules in hospital settings. However outside hospital settings there is also an increase in patients having infections with antimicrobial resistant strains. This is partially attributed to an adaptation of organisms involved in nosocomial infections (e.g. Hospital versus Community Associated MRSA). However, a source for these organisms can also be livestock production and food thereof.

1.2.2 Complex epidemiology of ESBL-producing bacteria

ESBL-genes that confer resistance to almost all beta-lactam antibiotics are often located on mobile genetic elements (plasmids). Beside the survival and spread of successful ESBL-producing strains (clonal spread), the plasmids enable ESBL-genes to transfer horizontally from one bacteria to another, spreading resistance between bacteria and even between bacterial species. This is a complete different epidemiology as for instance for MRSA, where only the strain itself is important for the spread of the resistance mechanism. This complex epidemiology of strains, plasmids and resistance genes makes it more difficult to link human cases with ESBL-infections to a certain reservoir.

Predominant ESBL-types and plasmids in human and animal sources
The potential contribution of ESBL producing bacteria in food-producing animals and foods to public health risks is related to specific plasmid-mediated ESBL genes encoded by a number of organisms. The predominant ESBL families in both animal and human enterobacteria are CTX-M, TEM, SHV, and CMY. The most common genes associated with this type of resistance in humans are *bla*$_{CTX-M-15}$ and *bla*$_{CTX-M-14}$. The most common genes associated with this type of resistance in animals are *bla*$_{CTX-M-1}$, *bla*$_{CTX-M-14}$, followed by *bla*$_{TEM-52}$, *bla*$_{SHV-12}$ and *bla*$_{CMY-2}$ (EFSA, 2011). Most publications on ESBL-producers in meat

or food focussed on *Salmonella* or commensal *E. coli*. A few investigations also included other Enterobacteriaceae, like *Enterobacter cloacae* or *Klebsiella pneumonia*, however information on the presence of ESBL-genes and plasmids in those species are scarce.

Clonal versus non-clonal transmission in humans and animals
Several studies show clonal spread is very important in ESBL-transmission. Those studies are mostly related to spread among humans An example is the clonal spread of *E. coli* ST131 carrying incFII plasmids with $bla_{CTX\text{-}M\text{-}15}$ (Rogers *et al.*, 2011). However spread between humans and animals is mostly non-clonal and determined by plasmid spread. As shown in a study in broilers and broiler farmers, similar plasmids and ESBL-genes can be found in epidemiologically unrelated strains in humans and animals. This was also shown in a study on $bla_{CTX\text{-}M\text{-}14}$ on incK plasmids found in isolates from humans, turkeys and cattle in England and Wales. Here it was concluded that incK plasmids were common vectors for horizontal dissemination of 30% of the $bla_{CTX\text{-}M\text{-}14}$ genes to different *E. coli* isolates from humans, cattle and turkey. Comparison of the *E. coli* genotypes carrying incK with $bla_{CTX\text{-}M\text{-}14}$ showed a diversity of *E. coli* strain types (Stokes *et al.*, 2012), confirming a non-clonal spread. Also in Dutch isolates derived from human patients and broilers revealed similar ESBL genes ($bla_{CTX\text{-}M\text{-}1}$, $bla_{TEM\text{-}52}$) on identical plasmid subtypes (IncI1 ST3/ST7/ST10) in human and broiler isolates and diverse *E. coli* genotypes were present in both reservoirs (Leverstein-van Hall *et al.*, 2011).
One study using whole genome sequencing (de Been *et al.*, 2013)and another international study using a micro array platform to compare strains (Wu *et al.*, 2013)generated similar results: identical ESBL-genes were present in human and poultry isolates, but all strains were genetically very diverse from each other. These studies confirm that spread of ESBL-genes takes mainly place via horizontal transmission of plasmids.

ESBL-producing strains are able to spread vertically by clonal distribution or horizontally by spread of ESBL genes through plasmid conjugation between bacterial species. The type of spread depends on and is related to the type of bacteria, type of ESBL gene and type of plasmid. In the spread of ESBL producing strains between animals and from animals to humans (or vice versa) the mechanism of horizontal spread of plasmids carrying ESBL genes seems most important.

1.2.3 Spread and prevalence in different reservoirs

Food and food-producing animals
In the Netherlands, monitoring of antimicrobial susceptibility of isolates derived from food-producing animals started in 1998. This monitoring program is based on non-selective culturing of indicator isolates (*E. coli*, and *Enterococcus* spp) and zoonotic bacteria (*Campylobacter* spp and *Salmonella* spp) derived from faeces or meat. Per flock of animals or meat product one isolate per bacterial species is cultured and its susceptibility to a panel of antibiotics is determined. In this way antimicrobial resistance can be monitored over time. This method is European wide applied, based on the Zoonoses Directive 2003/99/EC and European results are published every year by EFSA. This European Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2012 (2014), showed the presence of cefotaxime resistance in several reservoirs (faeces from poultry, pigs and cattle). Eight member states (including the Netherlands), reported the highest cefotaxime (3rd generation cephalosporin) resistance levels in isolates derived from poultry (Table 1.1). Traditionally the lowest level of resistance, regardless of source, is reported in Northern European countries, like Denmark, Sweden, Finland and Norway (Table 1.1).

Table 1.1

Resistance (% Res) to cefotaxime in indicator E. coli *isolates in MSs in 2012 testing in either poultry, pigs or cattle (2014)using non-selective isolation.*

Country	poultry		pigs		cattle	
	N	% Res	N	% Res	N	% Res
Austria	130	3.1	140	0	273	0.7
Belgium	325	28	205	2.9	364	8.8
Denmark	115	1.7	152	0.7	98	0
France	201	10.4	200	2	-	-
Germany	-	-	-	-	515	2.5
Finland	-	-	-	-	295	0
Hungary	105	7.6	68	1.5	-	-
Netherlands	**292**	**5.8**	**284**	**0**	**559**	**0.5**
Norway	113	0.9	-	-	-	-
Poland	328	10.7	190	2.6	190	2.6
Sweden	255	0.4	-	-	-	-
Switzerland	185	2.2	185	1.1	187	0.5

N= total number of isolates tested; % Res = proportion of isolates that was cefotaxime resistant

Although these data can be compared between countries and comparison over the years is possible, for ESBL-monitoring the non-selective culturing of isolates is less sensitive than selective isolation methods (in which a 3rd generation cephalosporin is already added to the growth medium).However data from selective culturing is not yet available from every European country or every food or animal category. This method will be implemented for the entire EU in 2015.

Since 2011, active surveillance of ESBL-producing isolates in isolates derived from veal calves, dairy cows and pigs takes place at the Central Veterinary Institute (CVI) together with the Netherlands Food Products and Safety Authority (NVWA) and the animal Health Service (GD) for layers. The results of this monitoring and the result of a pilot study among dogs and cats (Hordijk *et al.*, 2013) in 2012 is shown in Table 1.2. In this table the results are compared to data available from Sweden and Denmark that used a similar sensitive, selective isolation method to obtain the results. Although data is scarce, prevalence data that can be compared are mostly higher in the Netherlands than compared to the prevalence found in the different categories (slaughter pigs, dairy cows, dogs, beef, and pork or poultry meat) in Sweden and Denmark.

Table 1.2.

Prevalence (%) of ESBL-producing E. coli *in different animal (farm prevalence) or food categories in the Netherlands, Sweden and Denmark using selective isolation.*

	NL		SE		DE
	'12	'13	'12	'13	'12
veal calves	70	46		1	
slaughter pigs	75	57			8
dairy cows	8	7	1		10*
broilers	100*		49	40	27**
laying hens		55	13		
wild birds	13				
dogs	45/55#		1		
cats	0/25#				
beef (meat)	6	5			0
pork	1	2			0
mixed meat	7				
poultry meat	73	83	41	51	36
turkey meat	29	35			

*2011 data **2010 data, #healthy-diarrheic cats/dogs respectively
NL=the Netherlands (Hordijk *et al.*, 2013; Nethmap/MARAN, 2013, 2014)SE=Sweden (SWEDRES/SVARM, 2013, 2014), DE=Denmark (DANMAP, 2013).

Dutch broiler faeces samples were tested only in 2011. 100% of the broiler batches (29 batches with 10 animals each) contained positive animals (Nethmap/MARAN, 2012). This confirmed a study performed in 2009 at broiler farms where on all 26 farms ESBL-positive isolates were collected from broilers and on 85% of the farms the prevalence at the farm was ≥80% (Dierikx *et al.*, 2013). Preliminary (unpublished) data from January - July 2014 in which one sample per flock broilers is selectively cultured for ESBL-producing *E. coli* shows 181/265 (68%) ESBL-positive samples, which might suggest a decrease in the ESBL-prevalence in broilers. This is still higher as found in Sweden in 2012 (Table 1.2). However, it is unknown if this adequately reflects the prevalence at or within broiler farms.

In addition to the presence of ESBL producing isolates in meat, recently ESBLs were found to be present in fresh culinary herbs imported from South-East Asia (Veldman *et al.*, 2014). Other food products examined and found to be positive for ESBL-producers are raw egg surface and fresh salads in India (Rasheed *et al.*, 2014)and in 2010 in vegetables sold in the Amsterdam area 5% of samples contained ESBL-producing Enterobacteriaceae (*Enterobacter cloacae, Citrobacter freundii and Klebsiella pneumonia*). These were found on parsnip, bean sprouts and radish (Reuland *et al.*, 2014).

ESBL-producing strains are found in Dutch broilers, pigs, veal calves and dairy cows and meat thereof. The percentage of positive animals is highest in broilers (68%-100%) and lower in pigs, veal calves and dairy cows (57, 46 and 7%). During the last three years ESBL levels in animals in the Netherlands tend to decrease.
ESBL-producing bacteria are found are also found in dogs (45 – 50%), incidentally in cats, in the environment (water), wild birds, and vegetables.

The environment

Other Dutch data on ESBL-prevalence includes ESBLs in flies at poultry farms. Two pools (one of three blow flies and one of eight house flies) out of nineteen pools (containing 1 to 8 flies) of flies were positive for ESBL-producing *E. coli* and the strain types, ESBL-genes and plasmids turned out to be similar as found in the environment (manure/rinse water) of the farm (Blaak *et al.*, 2014b).

In addition, a recent study by the National Institute for Public Health and the Environment (RIVM) on ESBL-producing isolates on fresh products (vegetables) and agricultural soil showed mainly the presence of strains that are intrinsically resistant to 3rd generation cephalosporins and no ESBL-producing *E. coli* were found. Future studies are planned to determine the amount of ESBL-producing isolates and calculate the level of exposure to humans (Blaak *et al.*, 2014b).

A study on prevalence and characteristics of ESBL-producers in Dutch recreational waters and how this was influenced by wastewater treatment plants found that ESBL producing *E. coli* are present in recreational waters. It was concluded that wastewater treatment plants were not the only source for these bacteria (Blaak *et al.*, 2014a).

1.2.4 Quantity of ESBLs in different sources

Quantitative data on the presence of ESBL-producers is sparse. In the Netherlands this information was collected in a study on broiler meat (Cohen Stuart *et al.*, 2012). On broiler meat the median loads of ESBL-producing isolates were 80 colony forming units (cfu)/25 g meat in conventional meat samples versus <20 cfu/25 g meat in organic samples. In a not yet published study on ESBL-producing *E. coli* in broilers on one broiler farm, the amounts of ESBL-producers varied between 10 cfu/g – 10^7 cfu/g faeces. The proportion ESBL-producing *E. coli* versus non-ESBL-producing *E. coli* varied between 1:10 to 1:10.000.

Quantification of ESBL-producing *E. coli* in pig samples in a longitudinal study was performed in Denmark (Hansen *et al.*, 2013).They saw a decrease in cfu counts corresponding to older age of the piglets. Piglets, weaners and finishing pigs had average counts of ESBL-producing isolates of 10^7, 10^5 and 10^3 cfu/gram faeces respectively. In an earlier study in the UK, the amount of ESBL-producers was described in cattle, chicken and pig faeces in the UK. The average counts for ESBLs in chicken and pig faeces were higher than in cattle faeces (8.6 10^4 cfu/g in cattle faeces compared to 2.1 10^5 and 1. 10^5cfu/g in chicken en pig faeces), but all were considered high density shedders (> 10^4 cfu/g faeces).

Until now it is unknown at what levels the presence of ESBL-producing *E. coli* in food-producing animals poses an increased risk to contaminate carcasses in the slaughterhouse, survive meat processing and more importantly, and to contaminate food products in supermarkets. This may even vary by animal species, given the differences in slaughter and meat processing systems.

In recreational waters concentration of ESBL-producing *E. coli* ranged from 0.15 to 15 cfu/100 ml water. ESBL-producers represented 0.05-1% of the total *E. coli* population in positive water samples. Concentration of ESBL-*E. coli* at waste water treatment plants discharge points were on average 2- to 3-$\log_{10}$ units higher than that in recreational waters (Blaak *et al.*, 2014a).

At the moment no data is available to determine which amount of ESBL-producing organisms or ESBL-genes in food or environment will result in effective transmission to humans or human pathogens.

Quantitative info on ESBL presence in different reservoirs is scarce. Information about the minimum dose of ESBL-producing bacteria that can result in colonization of the human gut is lacking. Moreover, the chance of infection in humans that are colonised with ESBL-producing bacteria in their gut is currently unknown.

1.2.5 Risk factors for ESBL-presence in food-producing animals

The use of antimicrobials is reported in several scientific studies as being a risk factor that selects for resistant bacteria. Cavaco et al., (Cavaco *et al.*, 2008) studied in an *in vivo* experiment the effect of amoxicillin, ceftiofur or cefquinome on the persistence of a CTX-M producing *E. coli* strain. Higher amounts of the resistant strain were found up to 22 days after discontinuation of the treatment in the treatment groups compared to the untreated control group. This effect persisted longer than the withdrawal time recommended for these antimicrobials and was more significant for ceftiofur (3rd generation cephalosporin) and cefquinome (4rd generation cephalosporin) than for amoxicillin. Another publication describes the effect of a voluntary ban on cephalosporin use in Danish pig production in the reduction of ESBL-producing *E. coli* in slaughter pigs. The occurrence of ESBL-producing *E. coli* in pigs at slaughter was determined in 2009, 2010 and 2011. From July 2010, cephalosporin consumption in Danish pig production was almost zero. Prevalence of ESBL-producing *E. coli* differed significantly in 2010 and 2011 (11.8% compared to 3.6% respectively). Also a significant reduction of ESBL-producers at pig farm level was observed (11% of the farms positive in 2010 and 0% in 2011) (Agerso and Aarestrup, 2013). Also in the Netherlands data is available that supports the hypothesis that antimicrobial use influences the spread and persistence of resistant bacteria. From 2003 till 2010, data from the Dutch monitoring program on antimicrobial resistance in food-producing animals shows an increase in cefotaxime resistance in *E. coli* and *Salmonella* isolates derived from Dutch broilers. It became clear that although 3rd generation cephalosporins were not allowed in poultry production, they were used to prevent disease in one-day old chicken and ceftiofur was even sprayed over one-day old broilers in the hatcheries.

A total ban on the usage of ceftiofur in Dutch hatcheries in 2010 has resulted in a decrease in resistance to 3rd generation cephalosporins in the years thereafter (Nethmap/MARAN, 2014). In addition to the ban of ceftiofur at hatcheries, an enormous decline in antibiotic use in the food producing animal sector was established in the Netherlands. In 2013 antibiotic usage had declined with 63% compared to the top year of antimicrobial use 2007. The Dutch government recommended a reduction of 50% in 2013 compared to 2009 as index year. This target was indeed reached with a reduction of 58% in 2013 compared to 2009. As a result antimicrobial resistance levels, especially those from commensal *E. coli* has declined in all animal groups (Nethmap/MARAN, 2014).

In a study on risk factors for ceftiofur resistance in *E. coli* from Belgian broilers also the use of antimicrobials (specifically the beta-lactam antibiotic amoxicillin) was found to be one of the risk factors for a high level of ceftiofur resistant *E. coli* on the farm (Persoons *et al.*, 2011). However other risk factors were also found: poor hygienic condition of the medicinal treatment reservoir, no drinking water acidification, more than three feed changes per production cycle, hatchery, breed, and litter material. In a Swiss study on ESBL-occurrence in calves younger than 2 years, faecal carriage of ESBL producing Enterobacteriaceae was higher in calves (1) derived from dairy producing farms, compared to meat producing farms, (2) that derived from a farm that had more than one animal movement per day per 100 animals compared to a farm that had less than one animal movement per day per 100 animals and (3) that were less than 6 months of age compared to older calves (Reist *et al.*, 2013). Risk factor 1 and 3 were most likely indirectly related to antimicrobial use (mainly 3rd and 4th generation cephalosporins) on the farm of origin. On Swiss dairy farms, the calves that go for slaughter are either fattened on their farm of birth or they are sold to fattening farms at a very young age. Those fattened on the dairy farms are primarily fed with milk, including waste milk that cannot be put on the market because of elevated cell counts or previous antimicrobial use. Calves fed on fattening farms leave their dairy farms at very young age, therefore the chance is lower that they are exposed to milk contaminated with antimicrobials (Reist *et al.*, 2013). In comparison to Swiss dairy farms 3rd and 4th generation cephalosporins are less predominantly used in Swiss beef and fattening farms. Risk factor 2 seems also logic.

As already mentioned, the organization of the veal calve industry can be compared to an inverted pyramid. Calves are transported and collected from many different places even many different countries. As a result calves of different origin are placed together on a farm. This enhances the chance to import resistant strains from other places than the farm on which they will grow until slaughter. This was also confirmed by a study in which was shown that operating a closed farm policy reduced the risk of the dairy farm having ESBL *E. coli* compared to dairy farms that were open and did not quarantine

new cattle ((Snow *et al.*, 2012). In addition this last study performed in a geographical region in North West England and North Wales revealed three other risk factors for the presence of CTX-M-positive *E. coli* on dairy farms. (1) The use of 3rd or 4th generation cephalosporins on the farm during the last 12 months; (2) the storage of slurry in a pit as opposed to storage in a tank; and (3) disinfection of calf equipment less than once a month opposed to disinfection more often than once a month (Snow *et al.*, 2012). In this study selective culturing was used to detect ESBL-presumptive *E. coli*.

Another indirect association of the influence of the presence of resistant bacteria in the animal reservoir to the presence in humans is given by a study in Canada. After a voluntary withdrawal of ceftiofur in chicken hatcheries in 2005 a significant decrease in ceftiofur resistance was observed in bacteria from retail chicken and humans. After reintroduction of use, increasing levels of extended spectrum cephalosporin resistance were observed in bacteria from chicken and humans (Dutil *et al.*, 2010).
The use of antimicrobials, dissemination of ESBL-producers through animal movements and vertical spread within production chains are mentioned as the most important risk factors correlated to the occurrence, emergence and spread of ESBL/AmpC producing bacteria in a scientific opinion described by the BIOHAZ panel of EFSA (2011).

An important risk factor for the presence of ESBL-producers in animals is the use of antimicrobial agents. Use of cephalosporins and broad spectrum beta-lactams will directly select for ESBL-producing bacteria. Because ESBL-producing bacteria are often multi-drug resistant, also the use of other antibiotics may indirectly have a co-selective effect. This association is well documented in several studies. Besides antibiotic use, other possible risk factors (e.g. transport of contaminated animals or animal products, insufficient hygiene on farms and in production chains, feed and water quality) will differ between animal production sectors. The number of reliable studies to determine other risk factors is still very limited.

1.3 Risks for human health

1.3.1 Animal to human transmission (direct and indirect)

A few publications indicate ESBL transfer via direct contact to humans. By plasmid typing, it was shown for Dutch broiler farmers that two out of 18 farmers shared similar ESBL-genes located on genetically related plasmids with their broilers. All similar ESBL genes and plasmids were found in a variety of *E. coli* isolates, indicating a non-clonal spread (Dierikx *et al.*, 2013). Similar results were found in Denmark where ESBL-gene $bla_{CTX\text{-}M\text{-}1}$ was found in isolates from farm workers, pigs, air samples and manure located on genetically highly similar incN plasmids (Moodley and Guardabassi, 2009), but again the strains that contained the plasmids differed between different sources. These genetic associations indicate the possible transfer of ESBL-carrying plasmids from an animal reservoir to humans via direct contact.
Recently, clonal spread of ESBL *E. coli* from broilers to farm workers or family of the farmers has also been documented (Huijbers *et al.*, 2014). In this study 50 farms and people working and/or living on 47 of these farms (n=141) were sampled. On five farms clonal spread was found from ESBL containing *E. coli* found in broilers and farmer or family members on the farm. Human prevalence was 19.4% in this study (15.5% among farmers, 37.5% among employees, 11.4% among partners and 15.7% among family members). On 12 farms the human and broiler isolates shared similar ESBL genes and phylogenetic groups within the same farm. Risk factors for humans contracting ESBL producing isolates included: having close contact (especially spending more hours in the poultry house), having diabetes or a skin disease, or sampling in July-December 2010.
Indirect evidence for transmission from animals to humans is shown by different studies. One study in the Netherlands compared ESBL-genes found in isolates derived from human rectal swabs, human blood cultures and chicken meat. The distribution of ESBL-genes found in the isolates from human rectal swabs and from the chicken meat isolates showed more similarity than the genes found in human blood cultures (see fig 1, (Overdevest *et al.*, 2011)). The study of Overdevest et al., suggests that two distinct ESBL-epidemiology's exist: one in the hospital and one in food producing animals.

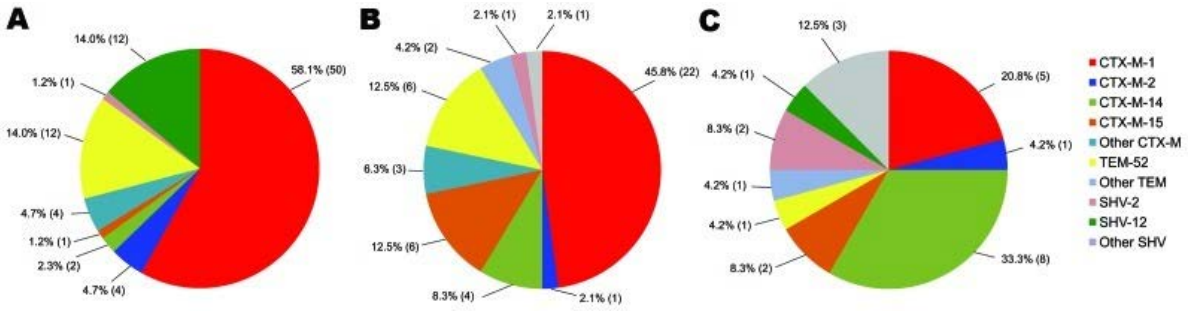


Figure 1.1 *Distribution of extended-spectrum beta-lactamase genes in chicken meat (A), human rectal swabs (B) and human blood cultures (C) in the Netherlands. Values in parentheses are no. positive (Overdevest et al., 2011).*

Direct contact of people with animals carrying ESBL-producing bacteria increases the chance of colonization of humans. Possible sources of plasmid located ESBL genes for humans are food, but also the environment. The majority of the types of ESBL genes and plasmids found within hospitals are different from those found in food producing animals. This indicates the presence of two distinct ESBL-epidemiology's: one in the hospital and one in food producing animals. A minority of the genes and plasmids that occur in ESBL-producing isolates humans in Dutch hospitals are genetically associated with genes and plasmids from poultry and poultry products. For other food animal species this genetic association is only demonstrated for genes and plasmids in isolates from farmers and the animals on the farm.

1.3.2 Transmission to humans from the environment

Indirect transmission from animals (broilers) via the environment to people living close to farms does not seem to play a major role. This was shown in a study by Huijbers *et al.*, in which individuals in areas with high broiler densities were found not to be at greater risk for ESBL carriage than people living in areas with low broiler densities (Huijbers *et al.*, 2013). There is no data available on the occurrence of ESBL-transmission to humans bathing in recreational waters. The amount of people in contact with recreational water will be higher for instance than the amount of people being in direct contact with livestock. However, the risk this poses for ESBL-transmission to humans is still unknown, but recreational waters should be considered as a potential exposure route to humans (Blaak *et al.*, 2014a).

Indirect ESBL transmission from the environment to humans might not play a significant role. However, the presence of ESBL-producers in relatively high counts in recreational waters might indicate recreational waters as a potential exposure route to humans. Causal evidence for this is lacking.

1.3.3 Risk factors for acquiring ESBL-producing bacteria from non-human sources

Recently a German study performed to asses risk factors associated with a community-acquired colonization of ESBL-producing *E. coli* resulted in the finding of being a native speaker of an Asian language and frequently consuming pork meat as independent risk factors for the colonization of ESBL-producers (Leistner *et al.*, 2013). However an additional study including German vegetarians couldn't find differences in ESBL colonization in vegetarians and meat-eating control persons (Meyer *et al.*, 2012). One of the conclusions from a risk profile on antimicrobial resistance transmissible from food animals to humans assessed by the RIVM on ESBLs was that there is sufficient evidence for an association between plasmids and the resistance genes they carry in human clinical isolates and in poultry isolates. However, the number of studies showing consistent molecular or epidemiological associations is at present too small to conclude that there is a causal relationship (RIVM, 2010).

1.4 References

2011, EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on the public health risks of bacterial strains producing extended-spectrum beta-lactamases and/or AmpC beta-lactamases in food and food-producing animals. EFSA Journal 9(8), 1-95.

2014, The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2012 EFSA Journal 12(3).

Agerso, Y., Aarestrup, F.M., 2013, Voluntary ban on cephalosporin use in Danish pig production has effectively reduced extended-spectrum cephalosporinase-producing Escherichia coli in slaughter pigs. The Journal of antimicrobial chemotherapy 68, 569-572.

Blaak, H., de Kruijf, P., Hamidjaja, R.A., van Hoek, A.H., de Roda Husman, A.M., Schets, F.M., 2014a, Prevalence and characteristics of ESBL-producing *E. coli* in Dutch recreational waters influenced by wastewater treatment plants. Vet Microbiol 171, 448-459.

Blaak, H., Hamidjaja, R.A., van Hoek, A.H., de Heer, L., de Roda Husman, A.M., Schets, F.M., 2014b, Detection of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* on flies at poultry farms. Applied and environmental microbiology 80, 239-246.

Cavaco, L.M., Abatih, E., Aarestrup, F.M., Guardabassi, L., 2008, Selection and persistence of CTX-M-producing *Escherichia coli* in the intestinal flora of pigs treated with amoxicillin, ceftiofur, or cefquinome. Antimicrobial agents and chemotherapy 52, 3612-3616.

Cohen Stuart, J., van den Munckhof, T., Voets, G., Scharringa, J., Fluit, A., Hall, M.L., 2012, Comparison of ESBL contamination in organic and conventional retail chicken meat. International journal of food microbiology 154, 212-214.

DANMAP 2013. DANMAP 2012. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. www.danmap.org.

de Been, M., Scharinga, J., Du, Y., Hu, J., Liu, Z., Lei, Y., Cen, Z., Cohen Stuart, J.W.T., Fluit, A., Leverstein-van Hall, M.A., Bonten, M.J., Willems, R., van Schaik, W., 2013. Whole-genome sequencing as a tool to determine whether dissemination of extended-spectrum beta-lactamase-producing *Escherichia coli* occurs through the food chain. In: ECCMID, Barcelona.

Dierikx, C., van der Goot, J., Fabri, T., van Essen-Zandbergen, A., Smith, H., Mevius, D., 2013, Extended-spectrum-beta-lactamase- and AmpC-beta-lactamase-producing *Escherichia col*i in Dutch broilers and broiler farmers. The Journal of antimicrobial chemotherapy 68, 60-67.

Dutil, L., Irwin, R., Finley, R., Ng, L.K., Avery, B., Boerlin, P., Bourgault, A.M., Cole, L., Daignault, D., Desruisseau, A., Demczuk, W., Hoang, L., Horsman, G.B., Ismail, J., Jamieson, F., Maki, A., Pacagnella, A., Pillai, D.R., 2010, Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. Emerging infectious diseases 16, 48-54.

Hansen, K.H., Damborg, P., Andreasen, M., Nielsen, S.S., Guardabassi, L., 2013, Carriage and fecal counts of cefotaxime M-producing *Escherichia coli* in pigs: a longitudinal study. Applied and environmental microbiology 79, 794-798.

Hordijk, J., Schoormans, A., Kwakernaak, M., Duim, B., Broens, E., Dierikx, C., Mevius, D., Wagenaar, J.A., 2013, High prevalence of fecal carriage of extended spectrum beta-lactamase/AmpC-producing Enterobacteriaceae in cats and dogs. Frontiers in microbiology 4, 242.

Huijbers, P.M., de Kraker, M., Graat, E.A., van Hoek, A.H., van Santen, M.G., de Jong, M.C., van Duijkeren, E., de Greeff, S.C., 2013, Prevalence of extended-spectrum beta-lactamase-producing Enterobacteriaceae in humans living in municipalities with high and low broiler density. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 19, E256-259.

Huijbers, P.M., Graat, E.A., Haenen, A.P., van Santen, M.G., van Essen-Zandbergen, A., Mevius, D.J., van Duijkeren, E., van Hoek, A.H., 2014, Extended-spectrum and AmpC beta-lactamase-producing *Escherichia coli* in broilers and people living and/or working on broiler farms: prevalence, risk factors and molecular characteristics. The Journal of antimicrobial chemotherapy.

Leistner, R., Meyer, E., Gastmeier, P., Pfeifer, Y., Eller, C., Dem, P., Schwab, F., 2013, Risk factors associated with the community-acquired colonization of extended-spectrum beta-lactamase (ESBL) positive *Escherichia coli*. an exploratory case-control study. PloS one 8, e74323.

Leverstein-van Hall, M.A., Dierikx, C.M., Cohen Stuart, J., Voets, G.M., van den Munckhof, M.P., van Essen-Zandbergen, A., Platteel, T., Fluit, A.C., van de Sande-Bruinsma, N., Scharinga, J., Bonten, M.J., Mevius, D.J., National, E.s.g., 2011, Dutch patients, retail chicken meat and

poultry share the same ESBL genes, plasmids and strains. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 17, 873-880.

Meyer, E., Kola, A., Schiller, R., Schwab, F., Gastmeier, P., 2012. ESBL and VRE colonisation in attendees of an infection control symposium. In: European Congress of Clinical Microbiology and Infectious Diseases (ECCMID).

Moodley, A., Guardabassi, L., 2009, Transmission of IncN plasmids carrying blaCTX-M-1 between commensal Escherichia coli in pigs and farm workers. Antimicrobial agents and chemotherapy 53, 1709-1711.

Nethmap/MARAN 2012. Nethmap/MARAN 2012. Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2011, Central Veterinary Institute (CVI) of Wageningen UR, Lelystad. www.cvi.wur.nl/NL/publicaties/rapporten/maranrapportage/default.html.

Nethmap/MARAN 2013. Nethmap/MARAN 2013. Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2012, Central Veterinary Institute (CVI) of Wageningen UR, Lelystad. www.cvi.wur.nl/NL/publicaties/rapporten/maranrapportage/default.html.

Nethmap/MARAN 2014. Nethmap/MARAN 2014. Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2013, Central Veterinary Institute (CVI) of Wageningen UR, Lelystad. www.cvi.wur.nl/NL/publicaties/rapporten/maranrapportage/default.html.

Overdevest, I., Willemsen, I., Rijnsburger, M., Eustace, A., Xu, L., Hawkey, P., Heck, M., Savelkoul, P., Vandenbroucke-Grauls, C., van der Zwaluw, K., Huijsdens, X., Kluytmans, J., 2011, Extended-spectrum beta-lactamase genes of Escherichia coli in chicken meat and humans, The Netherlands. Emerging infectious diseases 17, 1216-1222.

Persoons, D., Haesebrouck, F., Smet, A., Herman, L., Heyndrickx, M., Martel, A., Catry, B., Berge, A.C., Butaye, P., Dewulf, J., 2011, Risk factors for ceftiofur resistance in *Escherichia coli* from Belgian broilers. Epidemiol Infect 139, 765-771.

Rasheed, M.U., Thajuddin, N., Ahamed, P., Teklemariam, Z., Jamil, K., 2014, Antimicrobial drug resistance in strains of *Escherichia coli* isolated from food sources. Revista do Instituto de Medicina Tropical de Sao Paulo 56, 341-346.

Reist, M., Geser, N., Hachler, H., Scharrer, S., Stephan, R., 2013, ESBL-producing Enterobacteriaceae: occurrence, risk factors for fecal carriage and strain traits in the Swiss slaughter cattle population younger than 2 years sampled at abattoir level. PloS one 8, e71725.

Reuland, E.A., Al Naiemi, N., Raadsen, S.A., Savelkoul, P.H., Kluytmans, J.A., Vandenbroucke-Grauls, C.M., 2014, Prevalence of ESBL-producing Enterobacteriaceae in raw vegetables. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology.

RIVM 2010. Risk Profile on antimicrobial resistance transmissable from food animals to humans, P.L. Geenen, M.G.J.K., H. Blaak, A.H. Havelaar, A.W. van de Giessen, ed. (RIVM).

Rogers, B.A., Sidjabat, H.E., Paterson, D.L., 2011, Escherichia coli O25b-ST131: a pandemic, multiresistant, community-associated strain. The Journal of antimicrobial chemotherapy 66, 1-14.

Snow, L.C., Warner, R.G., Cheney, T., Wearing, H., Stokes, M., Harris, K., Teale, C.J., Coldham, N.G., 2012, Risk factors associated with extended spectrum beta-lactamase Escherichia coli (CTX-M) on dairy farms in North West England and North Wales. Preventive veterinary medicine 106, 225-234.

Stokes, M.O., Cottell, J.L., Piddock, L.J., Wu, G., Wootton, M., Mevius, D.J., Randall, L.P., Teale, C.J., Fielder, M.D., Coldham, N.G., 2012, Detection and characterization of pCT-like plasmid vectors for blaCTX-M-14 in Escherichia coli isolates from humans, turkeys and cattle in England and Wales. The Journal of antimicrobial chemotherapy 67, 1639-1644.

SWEDRES/SVARM 2013. SWEDRES/SVARM 2012. Use of antimicrobials and occurrence of antimicrobial resistance in Sweden. The Public Health Agency of Sweden and the National Veterinary Institute (SVA), Uppsala, Sweden. www.sva.se.

SWEDRES/SVARM 2014. SWEDRES/SVARM 2013. Use of antimicrobials and occurrence of antimicrobial resistance in Sweden. The Public Health Agency of Sweden and the National Veterinary Institute (SVA), Uppsala, Sweden. www.sva.se.

Veldman, K., Kant, A., Dierikx, C., van Essen-Zandbergen, A., Wit, B., Mevius, D., 2014, Enterobacteriaceae resistant to third-generation cephalosporins and quinolones in fresh culinary herbs imported from Southeast Asia. International journal of food microbiology 177, 72-77.

Wu, G., Day, M.J., Mafura, M.T., Nunez-Garcia, J., Fenner, J.J., Sharma, M., van Essen-Zandbergen, A., Rodriguez, I., Dierikx, C., Kadlec, K., Schink, A.K., Wain, J., Helmuth, R., Guerra, B., Schwarz, S., Threlfall, J., Woodward, M.J., Woodford, N., Coldham, N., Mevius, D., 2013, Comparative analysis of ESBL-positive Escherichia coli isolates from animals and humans from the UK, The Netherlands and Germany. PloS one 8, e75392.

2. Background on carbapenemase-producing bacteria

2.1 What are carbapenemase-producing bacteria?

Resistance to third generation cephalosporins by ESBL-producers is even more complicated by the emergence of resistance to carbapenems (like imipenem, ertapenem, meropenem). Carbapenems are considered last resort antibiotics often used to treat infections with a multi-drug resistant organisms, like ESBL-producers. These compounds are not used in food-producing animals. Acquired resistance for carbapenems in Gram negative bacteria is usually based on the uptake of genes that encode enzymes (carbapenemases) that degrades all beta-lactams including the carbapenems and often also aztreonam. Although resistance can also develop as a result of changes in membrane permeability leading to the loss of specific outer membrane porins (Nordmann *et al.*, 2011).

2.2 Important types of carbapenemase genes found in human health

Like ESBLs, many types of carbapenemase genes confer resistance with slight differences in affinity to certain beta-lactam antibiotics. The most important gene-families found in human isolates are the class B metallo-beta-lactamases such as Verona integrin-encoded metallo-beta-lactamase (VIM, to date 42 variants), New Delhi Metallo beta-lactamase (NDM, 12 variants) and IMP (48 variants), the class A beta-lactamases, such as *Klebsiella pneumoniae* Carbapenamase (KPC, 22 variants) and class D including OXA-carbapenemases such as OXA-48 ((2013)www.lahey.org/Studies/other.asp#table1, last accessed on 23th of September 2014).

2.3 Origin of carbapenemases

The first KPC-gene (KPC-2) was found in humans in 1996 in the east of the US and has been clonally spread globally via patients from there to Puerto Rico, China, Israel, Greece and Colombia and causing hospital outbreaks in European countries and South America (Nordmann *et al.*, 2011). The first IMP type (IMP-1) was described in humans in Japan in 1991. Since then IMP and also VIM-types of metallo-betalactamases have spread worldwide. They are now endemic in hospitals in Greece, Taiwan and Japan. NDM types of carbapenemases originate from the Indian continent. All human cases in Europe are related to either import by travel from India, Pakistan or Bangladesh or from the Balkan and the Middle East. These last two are considered secondary reservoirs of NDM-genes. In contrast to KPC, NDM is located on a highly mobile genetic element (transposon) that facilitates rapid transfer between bacteria. OXA-48 was firstly found in Turkey in 2003 in a human patient. OXA-48 has now been found worldwide including southern and eastern parts around the Mediterranean Sea and Africa. There is an increasing trend of identification of OXA-48 producers in humans in countries such as France, Germany, Spain, the United Kingdom and also the Netherlands through transfer of hospitalized patients from disease-endemic areas that are the source of hospital outbreaks(Nordmann *et al.*, 2011). One example is the outbreak in the Maasstad hospital of an OXA-48 producing *Klebsiella pneumonia* in the Netherlands (Dautzenberg *et al.*, 2014).

2.4 Spread of carbapenemase-producing bacteria

Carbapenemase producing isolates are mainly described in human patients. At this moment only a few studies reported about the occurrence of carbapenemase producing isolates in samples from animals or the environment, including aquatic environments like hospital sewage, wastewater treatment plants, lakes or rivers (KPC-2, GES-5, BIC-1, IMI-2, VIM-1, VIM-2, VIM-13, IMP-8, IMP-10, IMP-13, NDM-1 and OXA-23) in all kinds of Enterobacteriaceae and non-fermenters (Woodford *et al.*, 2014). Although carbapenem antimicrobials are not used in food-producing animals in the EU, resistance has occasionally been detected in bacteria carried by animals (VIM-1, OXA-48, OXA-23 and NDM-1) (Woodford *et al.*, 2014). Like other resistance genes, carbapenemase genes are also found in bacterial species obtained from remote environments, like Alaskan soil which was never inhabited by humans.
Reports on carbapenemase encoding genes present in food-producing animals are from France (*bla*$_{OXA-23}$ found in *Acinetobacter* in dairy cattle, (Poirel *et al.*, 2012)), China (*bla*$_{NDM-1}$ found in *Acinetobacter iwoffii* from a broiler (Wang *et al.*, 2012)) and in *Acinetobacter baumannii* from a pig farm (Zhang *et al.*, 2013), Germany (*bla*$_{VIM-1}$ in *Salmonella* Infantis and *E. coli* at a pig farm and in *Salmonella* Infantis at a broiler farm (Fischer *et al.*, 2012, 2013a).
Carbapenems are also recently found in companion animals in Belgium (*bla*$_{OXA-23}$ found in *Acinetobacter* in two horses (Smet *et al.*, 2012)), Germany (*bla*$_{OXA-48}$ in *E. coli* and *Klebsiella pneumonia* isolated from dogs (Stolle *et al.*, 2013)), and USA (NDM-1 producing *E. coli* from five dogs and one cat (Shaheen *et al.*, 2013) and in wildlife in Germany (NDM-1 producing *Salmonella* Corvallis from a black kite (Fischer *et al.*, 2013b)).
However the scarce information on this topic might be biased by the lack of screening activities in the past. Until recently carbapenems had not been included in the antibiotic panels of national surveillance programmes, nor in the panels of antibiotics used by veterinary diagnostic laboratories. Moreover screening for resistance to carbapenems has not yet been compulsory in official European Union (EU) surveillance activities. Therefore data on resistance to this class of antimicrobials in the EFSA Community Summary reports is lacking. However, in view of the great importance of the carbapenem compounds, one (meropenem) has been added to the panels of antimicrobials recommended for testing by member states of the European Union to improve surveillance for resistance (2012). Moreover selective isolation of carbapenemase producers will become compulsory in 2015 for the entire EU.
It is important to include carbapenems in existing monitoring programs on antimicrobial resistance in food-producing animals and food. In this way it is hopefully possible to identify the emergence of carbapenemase genes in non-human sources. Carbapenems are last resort antimicrobials used to treat serious infections in humans. Resistance to these compounds should carefully be monitored so that actions can be taken to prevent spreading of the genes/plasmids or strains when necessary.

2.5 Situation in the Netherlands

In the Netherlands, until now, carbapenemase producing isolates have only been found incidentally. There has been hospital outbreaks with *bla*$_{OXA-48}$ producing *Klebsiella pneumonia* (in 2011 in the Maasstad hospital, (Dautzenberg *et al.*, 2014)) and *bla*$_{KPC}$-producing *Klebsiella pneumonia* in 2013 in a nursery in Geertruidenberg. *bla*$_{VIM-2}$ positive *Pseudomonas aeruginosa* has been described in a nosocomial outbreak in 2008-2009 in the Erasmus University Medical Centre (Van der Bij *et al.*, 2011). A nationwide surveillance study for carbapenemase producing *Pseudomonas aeruginosa* was performed in 2010-2011 in the Netherlands, which identified the presence of VIM-producing *Pseudomonas aeruginosa* in eight hospitals, two burn wound centers, one long-term healthcare facility and in two community patients attending a general practitioner (Van der Bij *et al.*, 2012).

Until now in the Netherlands, there are no reports on the presence of carbapenemases in isolates from other reservoirs than humans except OXA-48, chromosomally encoded in environmental organisms like *Shewanella* (pers. Communication D. Mevius) and a OXA-51-like gene in *Acinetobacter baumanni* isolated from four dogs in a Dutch animal intensive care unit in 2012 (Leendertse *et al.*, 2013).

2.6 Risk factors for the presence of carbapenemase producing bacteria in food-producing animals

At the moment, in the Netherlands carbapenemase producing bacteria are only found sporadically in humans and pet animals. Because of the close interaction between pets and their owners and the use of modern broad-spectrum antimicrobials in companion animal health care, a possible introduction of carbapenemase producers in companion animals is more likely than in food-producing animals. If it will occur, the most likely route in which food-producing animals, foods and the environment can be affected is through transmission from humans (or pet animals) to livestock. This could occur either in the Netherlands or in other countries and can be introduced in the Netherlands by trade of contaminated animals or feed and food products. However, if carbapenemase producers are introduced in livestock it is possible that they will be selected by using any kind of antimicrobial as most of these isolates are multidrug resistant. In that way, although no carbapenems are used in Dutch livestock, they may be able to survive and spread due to the use of other compounds. Import from areas where carbapenemases are very commonly found (for example South-East Asia) are checked randomly by the NVWA (RIVM, 2014).

Until now, reports of the presence of carbapenemase producers is mainly restricted to reports in humans. Carbapenemases in isolates from food producing animals are described in isolated cases of animals in Germany, France and China. However the scarce information might be due to lack of resistance monitoring programs that had included carbapenem antibiotics. Because of the close interaction between pets and their owners and the use of modern broad-spectrum antimicrobials in companion animal health care, a possible introduction of carbapenemase producers in companion animals is more likely than in food-producing animals. However whenever this happens, import of carbapenemase producers in livestock will probably occur via human carriers. Due to their multi-resistant character, it is possible that in spite of the absence of direct selective pressure by use of carbapenemens, carbapenemase producers may survive and be transferred in the animal environment. Therefore in 2015, monitoring for carbapenem resistance in livestock will be compulsory in faeces of selected food-animal for the entire EU, including selective isolation of carbapenemase producers. In the Netherlands this program started in 2012 and will be continued on all fecal samples of food-animals collected per year (app. 1500/y).

2.7 References

2012, Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in *Salmonella*, *Campylobacter* and indicator *Escherichia coli* and *Enterococcus* spp. bacteria transmitted through food. EFSA Journal 10(6).

2013, EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on carbapenem resistance in food animal ecosystems. EFSA Journal 11(12).

Dautzenberg, M.J., Ossewaarde, J.M., de Kraker, M.E., van der Zee, A., van Burgh, S., de Greeff, S.C., Bijlmer, H.A., Grundmann, H., Cohen Stuart, J.W., Fluit, A.C., Troelstra, A., Bonten, M.J., 2014, Successful control of a hospital-wide outbreak of OXA-48 producing Enterobacteriaceae in the Netherlands, 2009 to 2011. Euro surveillance 19.

Fischer, J., Rodriguez, I., Schmoger, S., Friese, A., Roesler, U., Helmuth, R., Guerra, B., 2012, Escherichia coli producing VIM-1 carbapenemase isolated on a pig farm. The Journal of antimicrobial chemotherapy 67, 1793-1795.

Fischer, J., Rodriguez, I., Schmoger, S., Friese, A., Roesler, U., Helmuth, R., Guerra, B., 2013a, Salmonella enterica subsp. enterica producing VIM-1 carbapenemase isolated from livestock farms. The Journal of antimicrobial chemotherapy 68, 478-480.

Fischer, J., Schmoger, S., Jahn, S., Helmuth, R., Guerra, B., 2013b, NDM-1 carbapenemase-producing Salmonella enterica subsp. enterica serovar Corvallis isolated from a wild bird in Germany. The Journal of antimicrobial chemotherapy 68, 2954-2956.

Leendertse, M., Hordijk, J., Wagenaar, J.A., Robben, J.H., Broens, E., 2013. The first outbreak of a multidrug resistant Acinetobacter baumannii strain on a Dutch animal intensive care unit. In: 5th ARAE symposium 2013, Ghent, Belgium, p. 21.

Nordmann, P., Naas, T., Poirel, L., 2011, Global spread of Carbapenemase-producing Enterobacteriaceae. Emerging infectious diseases 17, 1791-1798.

Poirel, L., Bercot, B., Millemann, Y., Bonnin, R.A., Pannaux, G., Nordmann, P., 2012, Carbapenemase-producing Acinetobacter spp. in Cattle, France. Emerging infectious diseases 18, 523-525.

RIVM 2014. Advies Preventie en bestrijding van carbapenemresistentie in Nederland.

Shaheen, B.W., Nayak, R., Boothe, D.M., 2013, Emergence of a New Delhi metallo-beta-lactamase (NDM-1)-encoding gene in clinical Escherichia coli isolates recovered from companion animals in the United States. Antimicrobial agents and chemotherapy 57, 2902-2903.

Smet, A., Boyen, F., Pasmans, F., Butaye, P., Martens, A., Nemec, A., Deschaght, P., Vaneechoutte, M., Haesebrouck, F., 2012, OXA-23-producing Acinetobacter species from horses: a public health hazard? The Journal of antimicrobial chemotherapy 67, 3009-3010.

Stolle, I., Prenger-Berninghoff, E., Stamm, I., Scheufen, S., Hassdenteufel, E., Guenther, S., Bethe, A., Pfeifer, Y., Ewers, C., 2013, Emergence of OXA-48 carbapenemase-producing Escherichia coli and Klebsiella pneumoniae in dogs. The Journal of antimicrobial chemotherapy 68, 2802-2808.

Van der Bij, A.K., Van der Zwan, D., Peirano, G., Severin, J.A., Pitout, J.D., Van Westreenen, M., Goessens, W.H., Group, M.-P.S.S., 2012, Metallo-beta-lactamase-producing Pseudomonas aeruginosa in the Netherlands: the nationwide emergence of a single sequence type. Clinical microbiology and infection 18, E369-372.

Van der Bij, A.K., Van Mansfeld, R., Peirano, G., Goessens, W.H., Severin, J.A., Pitout, J.D., Willems, R., Van Westreenen, M., 2011, First outbreak of VIM-2 metallo-beta-lactamase-producing Pseudomonas aeruginosa in The Netherlands: microbiology, epidemiology and clinical outcomes. International journal of antimicrobial agents 37, 513-518.

Wang, Y., Wu, C., Zhang, Q., Qi, J., Liu, H., He, T., Ma, L., Lai, J., Shen, Z., Liu, Y., Shen, J., 2012, Identification of New Delhi metallo-beta-lactamase 1 in Acinetobacter lwoffii of food animal origin. PloS one 7, e37152.

Woodford, N., Wareham, D.W., Guerra, B., Teale, C., 2014, Carbapenemase-producing Enterobacteriaceae and non-Enterobacteriaceae from animals and the environment: an emerging public health risk of our own making? JAC 69, 287-291.

Zhang, W.J., Lu, Z., Schwarz, S., Zhang, R.M., Wang, X.M., Si, W., Yu, S., Chen, L., Liu, S., 2013, Complete sequence of the bla(NDM-1)-carrying plasmid pNDM-AB from Acinetobacter baumannii of food animal origin. The Journal of antimicrobial chemotherapy 68, 1681-1682.

3. How and where appear and spread ESBL-producing bacteria in the food chains?

3.1 Intro breeding pyramid

Chain structures and in particular breeding pyramid structures differ for pork, broiler and veal production. Appendix 2 gives insight in breeding pyramid structures for the selected animal production sectors.

Figure 3.1 provides a schematic representation of animal food production chains and possible transmission routes of veterinary risk to the chain and to plant environments. In the following paragraphs existing knowledge on the appearance and spread of ESBL-producing bacteria in the various stages of food production chains is presented and discussed.

3.2 Appearance and spread within feed mills

Purchase of raw material / production of concentrates
There are no studies known on prevalence of ESBL-producing bacteria in feed concentrates, raw feed materials and/or roughage. As the use of antibiotics in human and veterinary medicine as well as other applications has resulted in the widespread development of resistance also in the environmental reservoir (RIVM, 2010), contamination of crops used for animal feed production or used as roughage or bedding material cannot be excluded. However, further processing of raw materials into concrete feed might eliminate the contamination rate due to processing conditions (high temperature, pressure et cetera).

Contamination with ESBL-producing bacteria of crops used for animal feed production or used as roughage or bedding material cannot be excluded. Research data are lacking.

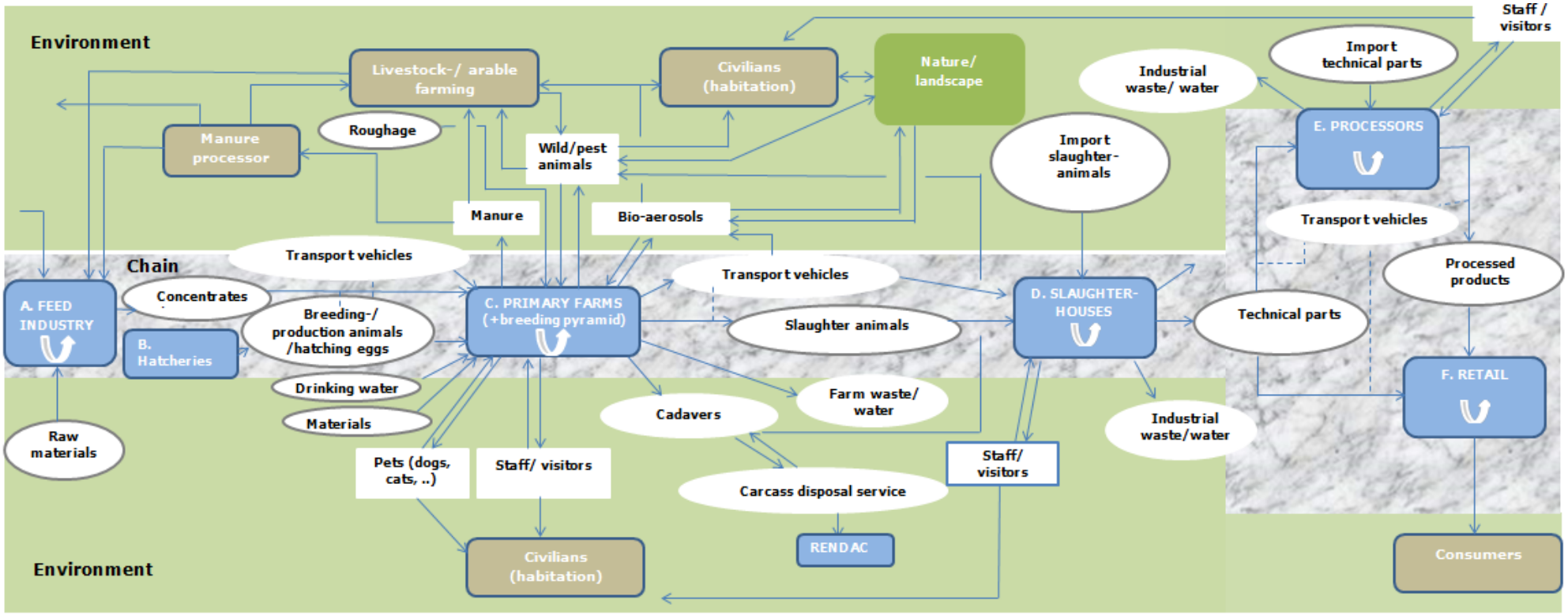
3.3 Appearance and spread within hatcheries (broilers)

The hatchery plays an important central role in the broiler poultry chain. Eggs from many diverse broiler breeder companies are transported towards the hatchery. Upon arrival at the hatchery, hatching eggs are stored for some days. Depending on the planning at the broiler farm, eggs are consequently unpacked from cardboard trays / transport trolleys and placed on incubator trays at incubator trolleys. Next, eggs are disinfected and placed into incubators. The incubation process lasts about 21.5 days, and the first 18 days eggs remain in setters. Three days before hatching the eggs are removed from the setters, and candled to exclude infertile eggs and eggs with dead embryos. Eggs containing live embryos are transferred into hatching baskets. Stacked hatching baskets are accordingly placed into hatchers, where the embryos find the right conditions to emerge from the eggshell and hatch. After 21.5 days of incubation, the hatcher doors are opened and the chicks are removed to the chick processing area. In this room, chicks are removed from the hatching baskets by hand or by means of automated equipment. The chicks finally are transported on belts towards the chick quality assessment and automated chick counters. Next, chicks are counted into chick transport boxes, vaccinated and stacked and placed into the chick holding area where they wait until the moment of transport towards the broiler farm. Logistically, all eggs and all chicks follow the same route through the hatchery. Consequently, each contact point, belt, incubator, basket, incubator or transfer area and ventilation channel can possibly attribute to contamination of batches of chicks with bacteria or viruses. With respect to the control of ESBL contamination in the broiler meat chain, it is important to control the broiler meat pyramid from the level of breeding company to the level of slaughter and retail (Dierikx *et al.*, 2013b; Laube *et al.*, 2013; Nilsson *et al.*, 2014; Persoons *et al.*, 2011). Despite the suspicion that broiler hatcheries may contribute to ESBL contamination of broilers investigations on the presence of ESBL producing

Escherichia coli in hatcheries have not yet been published. Preliminary results from a yet unpublished intervention study by Dierikx *et al* executed in 2013 in a Dutch commercial broiler hatchery showed that ESBL producing *Escherichia coli* bacteria were present in almost all phases of incubation in the hatcher. Only in the egg storage room and in the setter area (and in the setters) no positive samples could be detected (Dierikx *et al.*, 2014).
A total ban on ceftiofur usage in Dutch hatcheries in 2010, used for preventative treatment of 1-day-old chickens, has resulted in a decrease in resistance to 3rd generation cephalosporins in the years thereafter (see paragraph 1.2.5). Breeding and end chick hatcheries from March 2011 no longer use antibiotics at the hatchery. Hatcheries also no longer include antibiotics in deliveries of 1-day old chickens to breeding or broiler farms. Moreover, within the Chain Quality System (IKB) it is the broiler producer forbidden to use other veterinary drugs than those prescribed by his contracted veterinarian (http://www.pve.nl/wdocs/dbedrijfsnet/up1/ZckdpslIqC_MASTERPLAN-11-04-20-2011-M0022.pdf).

There are no studies published yet on contamination of hatcheries with ESBLs. Preliminary results show that ESBL-producing bacteria can be present in almost all phases of incubation in the hatcher. Dutch hatcheries has stopped using preventive antibiotics.

Figure 3.1 *Schematic overview of production plants in animal food chains and in their environment*



3.4 Appearance and spread within primary farms within different animal sectors

On primary farm level, several farm management processes are of interest related to the risks of entry and emergence of ESBLs, the internal spread and the transfer of ESBL-producers from primary farm to the next stage in the production chain. Primary farms have a significant role as amplifiers of resistance(2011).

Antibiotic use and farm health management

On-farm antibiotic use is considered an important factor in selection of AMR bacteria including ESBLs ((Dierikx *et al.*, 2013b; RIVM, 2010), see also paragraph 1.2.5). The use of antimicrobial drugs in animal healthcare and animal feed additives has contributed to the widespread development of resistance in farm animals and animal products *inter alia* (RIVM, 2010). In veterinary medicine, the increase in ESBLs shows parallels with the availability of third and fourth generation cephalosporins in the veterinary field. The prevalence of ESBL-positive birds in GPS breeding broilers stayed below 50%, except when beta-lactam antibiotics were administered: in that case the prevalence increased to 100% (Dierikx *et al.*, 2013b).

A huge decline in antibiotics use was established in the Netherlands: in 2013 a reduction of 58% was reached compared to 2009. In 2017, a reduction of 70% compared to 2009 is targeted. The amount of antibiotics used varies between production animal sectors and between farms within sectors. In 2013, the average DDDA's for white veal, rose veal, sows and piglets, fattening pigs and broilers were respectively 28.3 (white veal calves),10.8 (rose veal calves), 10.9 (sows and piglets), 5.7 (fattening pigs) and 14.7 (broilers).

Recent data suggest that exposure to antibiotics is not limited to administered dosages during treatment. Residues of antibiotics are frequently found in drinking water systems (Lamers *et al.*, 2012), faeces of food-producing animals and the farm environment (Berendsen *et al.*, 2015). The result is that animals frequently contain in their GI tract low concentrations of antimicrobials of different classes of which it is known that low sub-inhibitory concentration can select for resistant organisms in experimental settings (Gullberg *et al.*, 2011). Their effect under field conditions is unknown. Given the possible implications it is a priority to get more insight in the effect of antibiotics at residue levels in the farm environment and the GI-tract of food-producing animals.

Risk factors for on-farm antibiotic use

The quantity of antibiotics used on the farm is influenced by both the farm health status (e.g. the health management on the farm) and the farmer's mind-set: is he willing and capable to minimize the use of antibiotics. Paragraph 7.1 focusses on socioeconomic factors influencing the farmer's mind-set.

The on-farm animal health status as an important factor for prophylactic, curative and/or metaphylactic antibiotics use is a result of i) presence and circulation of infectious agents, ii) resistance and resilience of the animals against pathogens and iii) effective interventions taken when health problems arise ((Bokma *et al.*, 2014). Preventive measures and good animal health management will help reduce the (perceived) need for on-farm antibiotic use against pathogens and thus reduce selective pressure towards AMR and ESBL-producing bacteria.

E. coli are commensal bacteria in the gut: ESBL-producing bacteria are contagious but not infectious; they do not cause animal diseases themselves. Thus, animal resistance and resilience regarding these commensals is not at stake, the prevalence of ESBL-producing bacteria on primary farms depends on i) the selective pressure by on-farm antibiotic use and b) external and internal biosecurity measures taken to prevent introduction and spread of ESBL producing bacteria. Possible sources of introduction and/or spread of ESBLs are: purchased animals (or incubated eggs to be hatched on the broiler farm) and animal contacts between and within production groups; feed (concentrates, roughage or feed materials), drinking water and other materials (e.g. bedding); pest animals; staff/visitors; bio-aerosols in incoming and/or circulating air; and manure in animal houses/pens (hygiene). Existing knowledge on these issues is discussed in the following sections.

Animals

ESBL-producing bacteria can be introduced to (other) primary farms by purchased animals (veal calves, piglets/gilts/boars, day-old chicks or 18-day-incubated eggs (Patio-system broiler farms)).
Dierikx *et al* demonstrated the presence of ESBL/AmpC producing *E. coli* isolates at all levels in the broiler production pyramid (Dierikx *et al.*, 2013b). At the top of the pyramid the prevalence was lower than found at broiler production farms at the bottom of the pyramid. However, as there are worldwide only a few primary breeding companies at the top of the pyramid, resistance genes can easily spread in globally organized production systems. Mevius *et al.* (2009) also concluded in the MARAN-study that the risk of introducing ESBL/AmpC-producing *E. coli* in the broiler production chain occurs already at restocking of grandparent's flocks with positive chicks: ESBL- and/or AmpC-producing *E. coli* are introduced in the Dutch poultry production chain through imported day-old grandparent chickens(MARAN, 2011). Moreover the MARAN-data indicate that the occurrence of these organisms in the different levels of the Dutch poultry production chain is the result of amongst others vertical transmission and recirculation within farms. Hordijk et al, 2013. demonstrated that in young veal calves at farm arrival the prevalence of animals positive for *E. coli* producing different ESBL/AmpC genes in their faeces was substantially higher than after a number of weeks (Hordijk *et al.*, 2013). This suggests introduction of new ESBLs into veal calf farms by contaminated young dairy calves. The changes in prevalence during the first six weeks occurred while the animals were housed in individual pens with limiting contact between animals. The clonal distribution of multiresistant $bla_{CTX\text{-}M\text{-}14}$-producing *E. coli* variants that were also found on two unrelated farms suggests circulation in the veal-calf production system, possibly introduced to the farms in the past. These isolates were similar to those previously described in both animals and humans. Due to the small number of farms involved Hordijk et al. concluded that further study is required to assess the persistence of ESBL/AmpC-producing *E. coli* in veal calves, and to assess whether recolonization occurs within a production cycle or subsequent production cycles.

Feed

As stated already in paragraph 3.2, there are no studies known on prevalence of ESBL-producing bacteria in feed concentrates, raw feed materials and/or roughage. As the use of antibiotics in human and veterinary medicine as well as other applications has resulted in the widespread development of resistance also in the environmental reservoir (RIVM, 2010), contamination of crops used for animal feed production or used as roughage or bedding material cannot be excluded. Further processing of raw materials into concrete feed might eliminate the contamination rate due to processing conditions (high temperature, pressure etcetera). Michels *et al* studied under experimental conditions the incorporation of antimicrobial agents into vegetables from manured soil as well as microbiological effects due to contamination of food plants with ESBL-producing bacteria (Michels *et al.*, 2013). Several ESBL-producing *E.coli* were found in soil, roots and edible parts of one leek. They stated that it cannot be excluded that consumers of conventionally grown vegetables may be exposed to multi-resistant germs. In that case, these multi-resistant germs can also be present in crops that are used as roughage or otherwise in animal feed production.

In a study conducted by Dierikx *et al*, feed samples taken in the poultry house at broiler farms became contaminated with ESBL/AmpC producing *E. coli* after one or more production weeks (Dierikx *et al.*, 2013a). Contaminated feed pans can thus become a source of ESBLs spreading during the ongoing production period.

Water

Drinking water can be a major purveyor of animal diseases in barns. A study performed in 2012 by CVI Lelystad on 62 farms showed that on 18% of pig fattening, 24% of pig production (on average on 23% of pig farms) and 28% of broiler farms the drinking water was positive for ESBL-producing *E. coli.* (Personal communication D. Mevius)**.** An increased bacterial count at drink water supply level in the barn is indicative of the general drinking water hygiene. Pathogens like *S. aureus* (including MRSA), E*. coli* and *S. suis* but also harmful fungi and yeasts can grow or survive in water pipes. In the case of an insufficient water flow, biofilm can be formed in the pipes. Biofilm is a layer inside water pipes consisting of minerals, organic matter and microorganisms (bacteria, yeasts, fungi). The water flow thus can continuously become contaminated. Inadequate water pressure on drinking nipples can cause recoil of air (possibly contaminated with bacteria/residues of antibiotics) into the water pipe. Risk factors for

growth of biofilm are dead end pipes and (angle) curves. Extra biofilm growth will occur in 'dead' or not frequently used water pipes, such as separate drug tours (extra risk of production of AMR organisms). Extra biofilm forming can also occur in animal houses with higher environmental temperatures, such as farrowing pens.

Manure, bio-aerosols and other sources in animal houses
Theoretically, ESBL-producing bacteria can be introduced into farm houses by bio-aerosols in incoming air. The risk will depend on the number and characteristics of other farms in de neighbourhood, their farm prevalence regarding ESBL-producing bacteria and measures taken to reduce emission of bio-aerosols to the environment (see 4.2 Bio-aerosols/Spread to the environment). Furthermore, ESBL-producing bacteria could be spread on-farm by manure, dust and other sources present in animal houses and pens.

Laube *et al* showed that ESBL/AmpC-producing *E. coli* could be found in German broiler flock in various environmental samples of the animal housing during the first sampling (Laube *et al.*, 2013). These findings were similar to the findings in a study of Hiroi *et al* (Hiroi *et al.*, 2012) and they assumed that insufficient cleaning and disinfection can give rise to contaminated barns as a cause for high incidences of ESBL-producing bacteria in broiler farms. Dierikx *et al* also revealed that at broiler farms ESBL/AmpC producing *E. coli* were still present in the environment of the poultry house after cleaning and disinfection (Dierikx *et al.*, 2013b). Laube *et al* showed a significant increase in the detection of ESBL/AmpC-producing *E. coli* from the first to the second samplings in animal as well as in environmental samples (Laube *et al.*, 2013). They assumed enrichment of ESBL-producing bacteria in the course of a fattening period in animals and/or in their environment. Laube *et al* also stated that faeces, litter, and even dust may act as transmission sources of ESBL/AmpC-producing *E. coli* within a broiler barn; spreading of resistant bacteria may be due to the use of the same equipment, shoes, or clothes. They considered the large number of ESBL positive pooled faeces samples and dust samples evidence for a high relevance of environmental entities as a source of on-farm transmission of ESBL-producing bacteria (Laube *et al.*, 2013).

Bio-aerosols in livestock houses can contain high levels of microorganisms. They mainly originate from the animals themselves (faeces, urine, sneezing/coughing, respiration, skin particles, other animal (excretion) products like eggs, milk, and placenta), but may also originate from feed, bedding material, farmer, or from the incoming air. Bio-aerosols can be wet particles or dust particles. Generally, wet aerosols are directly dispersed in the air by sneezing, coughing or respiration. Because these droplets are very small (<100 µm)**,** they will evaporate very fast. After evaporation only the naked bacteria or viruses are left; these can be individual or clusters of micro-organisms. These micro-organisms are exposed to environmental factors (temperature, relative humidity, UV-light, oxygen) to a greater extent than micro-organisms that are enclosed in dust particles. (Diseased) animals are the main source of infectious bio-aerosols. A lot of viruses and bacteria survive well during some minutes in the air. Generally, viruses are less susceptible for demolition than bacteria.

Animals shed microorganisms mainly by means of faecal excretion, which may contain large amounts of microorganisms (Letellier *et al.*, 1999; Pell, 1997). The microorganisms in faeces can become airborne when dried faecal particles are disturbed by air flow or animal activity. Microorganisms in dry faeces that have low water content become airborne more easily than microorganisms in fresh faeces. Under typical livestock housing environmental conditions, it may take hours or days to dry the faeces to a water content less than 10% - which is the water content of airborne dust in livestock production systems (Aarnink *et al.*, 1999; Zhao *et al.*, 2013; Zhao *et al.*, 2011) tested three pig houses and found that more than 50% of the airborne bacteria were in the non-respirable range (particles larger than PM5 (> 5 µm), at inhalation not able to reach the alveoli and therefore in principle less harmful than smaller particles).

Litter is a mixture of bedding material (e.g. wood shavings, chopped straw, sawdust, and rice hulls etc.) animal faeces, dander and feed (Torok *et al.*, 2009). The provision of litter in livestock production systems may improve animal welfare by increasing the incidence of natural behaviours (Appleby and Hughes, 1991), which, however, may result in more microorganisms being present in the air than in housing systems without litter (Madelin and Wathes, 1989; Vucemilo *et al.*, 2007). The microorganisms can arrive in litter during the harvesting and processing of the bedding material, but

especially through animal excretion and secretion. Most of the bacteria in the poultry litter are Gram-positive. Gram-negative bacteria and mold account for a small fraction of the total microbial count, but due to the high concentration of the total microorganisms their numbers can still be high (Martin *et al.*, 1998). Lu *et al* found that non-pathogenic coliform bacteria (including non-pathogenic *E. coli*) were found at a rate of 250000 cfu/g faeces (Lu *et al.*, 2003).

Studies that tried to culture bacteria from the air in animal houses showed that the main fraction of airborne bacteria also consists of Gram-positive flora and only a small fraction contained Gram-negative bacteria (Zucker *et al.*, 2000).
Bakutis *et al.* (2004) reported that in terms of the total bacterial count, the proportion of Gram-negative bacteria was approximately 10% in cattle houses, 4.9% in pig houses, and 2.6% in poultry houses (Bakutis *et al.*, 2004). Zucker *et al* found that the airborne Gram-negative bacteria in pig and cattle houses are aerobic and include *Enterobacteriaceae*, *Pseudomonas spp.* and *Neisseria spp.*; no culturable obligate anaerobic Gram-negative bacteria were found (Zucker *et al.*, 2000). Possible reasons for the smaller proportion of airborne Gram-negative bacteria in livestock production systems are less excretion by animals than their counterparts and that these bacteria are more vulnerable to environmental stress such as oxidation, radiation, and dehydration, probably because of their thinner cell walls (Pal *et al.*, 2007; Theunissen *et al.*, 1993).

It is indicated that bio-aerosols in principle can contribute to spread of ESBL-producing bacteria (which are Gram negative) within livestock housing systems. It will depend on farm characteristics (e.g. species; amount of bio-aerosols produced; ESBL prevalence) and preventive measures taken (e.g. strictly separated climate systems between production groups; the use of air decontamination systems; hygiene) to what extent spreading of the resistant bacteria via bio-aerosols actually occurs.

Pest animals
Pest animals like wild birds, rodents and flies can be an important (transmission) source of all kinds of micro-organisms; including ESBL-producing bacteria (see 4.4). A professional on-farm control of pest animals is important to prevent introduction and spreading of ESBLs.

Staff/visitors
Paragraph 1.3.1 points out strong indications for transfer of ESBL-carrying plasmids from an animal reservoir to humans via direct contact. Humans can be infected by animal or human sources, and contaminated staff could in theory (re-)introduce a contamination with ESBL-producing bacteria to the animals.

Primary farms have a significant role as amplifiers of resistance. It is demonstrated that ESBL/AmpC producing *E.coli's* are present at all levels of the broiler production pyramid, with the highest levels of contamination at the bottom of the pyramid in the broiler farms. Systematic data on prevalences in the production pyramids of pigs and veal calves is lacking.
The prevalence of ESBL-producing bacteria on primary farms strongly depends on a) the selective pressure by on-farm antibiotic use and b) external and internal biosecurity measures taken to prevent introduction and spread of ESBLs. Information on the negative effects of frequently occurring antibiotic residues in farm environments and the GI-tract of animals is lacking. On farm, contaminated feed pans can become a source of ESBLs spreading during ongoing production periods. Drinking water on pigs and broiler farms have been found to be frequently positive for ESBL-producing *E. coli* and drinking water systems can become an amplifier and distributor of resistance. Insufficient cleaning and disinfection can result in highly contaminated barns and farm environment with ESBL-producing bacteria. Contaminated faeces, litter, bio-aerosols, as well as farm equipment etcetera can transfer and spread ESBLs within barns. Wild/Pest animals can spread all kinds of micro-organisms including ESBL-producing bacteria. Humans can be colonized by animal or human sources. However, colonized humans can also be a source of (re-)introduction of ESBL-producing bacteria, genes, or plasmids in the animals.

3.5 Occurrence and spread within slaughterhouse and meat processing plants

Slaughterhouses
ESBL-producing bacteria can be introduced at slaughterhouses by contaminated slaughter animals or, less likely, by slaughterhouse staff and personnel. Slaughter animals are purchased from Dutch farms or imported from abroad. It will depend on its prevalence in slaughter animals from the specific origins whether or not the risk of introduction of ESBL-producing bacteria to the slaughterhouse is substantial.

Horton *et al* studied the faecal carriage and shedding density of CTX-M ESBL-producing *E. coli* in cattle, chickens and pigs and its implications for food production (Horton *et al.*, 2011). They assumed that, with respect to faecal contamination, both the absolute levels as well as the proportion of CTX-M-resistant *E. coli* present are likely to be important factors for spread to the food chain. However, the relative importance of these two parameters (absolute levels and proportion of CTX-M-resistant *E. coli)* in contributing to risks of food contamination are not certain yet. As stated earlier in 1.2.4, it is unknown *at what levels* the presence of ESBL-producing *E. coli* in food-producing animals poses an increased risk to contaminate carcasses in the slaughterhouse, survive meat processing and to contaminate food products in supermarkets.

Swanenburg *et al* studied the role of the lairage in pig slaughterhouses as a potential source of *Salmonella* contamination of slaughtered pigs (Swanenburg *et al.*, 2001a). It was concluded that the waiting period in the lairage of 2h contains a substantial risk of contamination of slaughter pigs with *Salmonella*, especially pigs from Salmonella-free herds. Both the usual nor improved cleaning and disinfection were able to fully eliminate this risk. It is not known whether ESBL-producing *E.coli* in slaughter pigs pose a comparable risk of cross-contamination during lairage in slaughterhouses.
Since bacteria that could carry ESBL or AmpC genes are known as common inhabitants of the intestinal tract of animals, it is expected that they contaminate carcasses during the slaughter process. Any measure by which microbial contamination is reduced at slaughter, or during further processing and retailing will also indirectly help to contain the spread of ESBL/AmpC-producing bacteria to humans (2011).
In pig slaughterhouses a decrease in amount of ESBL-producing bacteria on carcass surfaces is expected due to the scalding and dehairing processes. Pigs are held for several minutes in a scalding tank at 45-60 degrees to loosen the hair. After scalding the pigs are mechanically dehaired by abrasion and singed in a gas flame to complete the hair removal process. Consequently, decapitation and opening of the carcass by cutting and removal of intestines and internal organs take place. This poses a risk for faecal contamination of the carcass and cross contamination of the slaughter line. Still, as shown by Namvar *et al*, the total *E. coli* counts from carcasses at the end of the slaughter process are decreased compared to the beginning of the process (Namvar and Warriner, 2006). Botteldoorn *et al* noticed regarding *Salmonella* in pigs a high degree of carcass contamination after slaughtering and concluded that slaughterhouse hygiene is a determinative factor for managing carcass contamination with *Salmonella* (Botteldoorn *et al.*, 2003). After splitting and cutting of carcasses in the further processing at the slaughterhouse, the half-carcasses are chilled or frozen and stored for further transport. The freezing process will further decrease the amount of ESBL-producing bacteria.

The expert panel used in the Dutch ESBL-attribution research project indicate that contamination of ESBL-producing bacteria on slaughter equipment and the slaughterhouse internal environment is likely (Personal communication D. Mevius, April 2014). However, during the slaughter process no multiplication of ESBL-producing bacteria is expected due to the low temperatures: no growth of ESBL-producing bacteria below 10 degrees Celsius. The expert panels consider spread of ESBL-genes by horizontal transmission of plasmids (conjugation) on slaughter equipment and meat to be not likely as well, as survival in the slaughter environment is to their opinion already an utmost challenge for the ESBL-producing bacteria: few available nutrients and no bacterial growth due to low temperatures.
Lassok and Tenhagen studied current literature on the worldwide presence of livestock-associated MRSA in various steps of the pork production chain and concluded that the slaughter process plays a decisive role in MRSA transmission from farm to fork (Lassok and Tenhagen, 2013). Superficial heat treatments

such as scalding and flaming during the slaughter process can significantly reduce the burden of MRSA on the carcasses. However, according to Lassok and Tenhagen recontamination with MRSA might occur via surface treating machinery, as a result of faecal contamination at evisceration, or via increased human handling during meat processing. They concluded that transmission of MRSA from pig to pork can be minimized by optimizing processes for carcass decontamination and avoiding recontamination by effective cleaning and personal hygiene management (Lassok and Tenhagen, 2013).

The veal slaughtering process differs from the pig process on one point: total removal of hide instead of scalding and dehairing. The poultry slaughtering process slightly differs from red meat slaughtering. There is no lairage, birds are received in crates, unloaded to holding areas and attached to conveyer belts and transported to the slaughter area. After stunning, killing and bleeding, the scalding takes place in a water bath with temperatures ranging between 50-60 degree Celsius. Feathers are mechanically abraded, the dehaired carcasses are spray washed and the carcass is opened, viscera et cetera are removed, the carcasses are chilled in a water bath, drained, packed and chilled or frozen.
Reich *et al* evaluated the presence of extended-spectrum ß-lactamase- and AmpC-producing Enterobacteriaceae in broiler chickens at slaughter (Reich *et al.*, 2013). ESBL-producers were found on 88.6% of the carcasses and 72.5% of the ceca. Most isolates were identified as *E. coli*. Reich *et al* conclude that meat processing contributes to overall transmission of bacteria from contamination during slaughtering and dressing, including transmission of resistant bacteria introduced at slaughterhouses by colonized animals onto the meat product. Reich et al consider the role of shedding of bacteria through faeces leading to carcass contamination evident in this study. The role of faeces is considered a vital point when assessing the transmission potential of ESBL-producers through the food chain.

Meat processing plants
ESBL-producing bacteria present on carcasses will be transported from slaughterhouses further into the food production chain. Meat processing plants purchase half-carcasses from Dutch slaughterhouses or import them from abroad. It depends on the prevalences in the half-carcasses from the specific origins whether or not the risk of introduction of ESBL-producing bacteria to the meat processing plant is substantial. Meat processing plants further process part of the purchased meat into pasteurised smoked and/or cooked meat products. The pasteurization, smoking and/or cooking process will most likely kills or reduces among others ESBL-producing bacteria present on the meat. Fresh not pasteurized, smoked, cooked or otherwise decontaminated meat and meat products therefore from this point on are considered to be the remaining potential sources of ESBL-producing bacteria that can be spread further to the remaining stages of the food chain (i.e. retail and consumers).

Staff/visitors
Paragraph 1.3.1 points out strong indications for transfer of ESBL-carrying plasmids from an animal reservoir to humans via direct contact. Humans can be contaminated by animal or human sources, and contaminated personnel of slaughterhouses or meat processing plants could (re-)introduce a contamination with ESBL-producing bacteria to the meat products. Wang *et al* studied the occurrence of MRSA in retail foods in China, the antimicrobial resistance and molecular characteristics of these strains (Wang *et al.*, 2014). The strain characteristics indicated that MRSA contamination in food could be from both animal and human origin and contamination with certain strains may be attributable to cross-contamination during slaughtering or food processing. To their opinion, contamination may originate from poor hygiene of workers during food preparation. This was not yet investigated for ESBL-producers. Lassok and Tenhagen also concluded that transmission of MRSA from pig to pork can be minimized by avoiding recontamination by effective cleaning and personal hygiene management in the slaughterhouse and during meat processing (Lassok and Tenhagen, 2013). Although this was not investigated specifically for ESBL producers, most likely will have an effect on contamination of meat with ESBL producers.

Slaughtering and meat processing are important factors in contamination of animal products with ESBL-producing bacteria. Heat treatments such as scalding and dehairing (e.g. in pig slaughtering) can significantly reduce the ESBL burden on carcasses. However, carcasses can become (re-)contaminated with ESBLs due to contaminated slaughter equipment (as a result of faecal contamination). The contamination rates of the final meat product can also be influenced by handling during meat processing. Effective cleaning and personal hygiene management in slaughterhouses are of main importance to achieve further reduction in ESBL-contamination of animal products, especially in broiler meat production.

3.6 Appearance and spread within retail

ESBL/AmpC producing isolates do occur on fresh meat in the supermarket. The most recent data on ESBL/AmpC producers on meat revealed the highest prevalence on poultry meat (83%). ESBL/AmpC producers were also found on turkey meat (35%), beef (5%) and pork (2%) (Nethmap/MARAN, 2014). How the meat was contaminated (during slaughter or afterwards during processing of the meat) is still unknown. Cross-contamination might play a role. In theory, besides contaminated animals also people that process the meat can be potential sources for the meat to be contaminated with resistant bacteria.

Within retail the highest prevalence of ESBL/AmpC producers is found on poultry meat (83%), with lower prevalences found on turkey meat (35%), beef (5%) and pork (2%). How and where, in which stage of the food production chain, the meat was contaminated is unknown.

3.7 Spread by transport vehicles

Animal transports
Transport of live animals can be regarded as the process of fasting (feed withdrawal at the farm), mixing of animals originating from various farms, the actual transport, unloading at the place of destination and regrouping of animals, and possibly the waiting time in the lairage before slaughter. During this process animals are subjected to different significant stress factors. These include the (social) stress imposed with handling during loading and unloading and possibly mixing with unfamiliar animals, feed withdrawal, and the transport characteristics such as duration, distance, driving skills, climate and stocking densities (Lo Fo Wong *et al.*, 2002; Warriss *et al.*, 1992). As a consequence, transport stress possibly leads to immunosuppression (Stanger *et al.*, 2005). Immunosuppression can influence the (need for) administration of antibiotics on the farm of destination (especially in the case of veal calves).

Cleaning and disinfection of trucks might be an issue. Previous research has shown that pigs can acquire *Salmonella* spp within 2 hours following exposure to a contaminated environment (Hurd *et al.*, 2001). In general, animals transported by haulier are more likely to travel with animals from other farms than those transported by the farmer (Mather *et al.*, 2007). Research in the Netherlands has shown that 80% of trucks transporting pigs were contaminated with *Salmonella* spp. before transportation, despite drivers being asked to clean and disinfect their trucks thoroughly before loading the pigs (Mannion *et al.*, 2008; Swanenburg *et al.*, 2001b). Showing that cleaning and disinfection of the trucks are not always optimal. Optimal cleaning and disinfection can significantly reduce the prevalence of *Salmonella* spp. found in trucks (Rajkowski *et al.*, 1998). This might also account for *E. coli* and any other bacteria. In the cleaning and disinfection procedure it is most important to include any tools such as brooms, tools for scraping faeces, boards for moving pigs and transport vehicles as well as areas to which the animals have no direct contact such as ante-rooms for changing clothes and boots and alleys for pig movements (Bode *et al.*, 2007).

Meat transports
No literature is found on the risk of cross contamination of meat during meat transports.

Not adequately cleaned and disinfected vehicles transporting live animals can be a source of contamination of animals with pathogens like *Salmonella* and *E. coli* due to faecal shedding. Data on contamination of live animals in transport vehicles with ESBLs are lacking. However, optimizing cleaning and disinfection procedures for transport vehicles including tools such as brooms, moving boards and faeces scrapers is considered to reduce the risk of cross contamination of animals with ESBLs.

3.8 Overall attribution of imports to NL-situation concerning ESBLs

Import of meat and live animals
Besides domestic ESBLs of animal origin there is also the risk that ESBLs are imported by animals or meat originating from outside the Netherlands.
Most transports between EU countries of live pigs are between neighbouring countries. Germany is the major importer, Denmark the major exporter of live pigs. Although the Netherlands has a large export within the EU and to third countries there is also a substantial import of animals. Animals that are here to be slaughtered (slaughter pigs and cattle) but also to be raised (veal calves). Meat that is imported can either be consumed in the Netherlands but also can be directly transported to other countries (transit) or after processing be exported. Although a large part of the products of imported animals as well as meat are exported, slaughtering and processing the animals poses the risk of introducing ESBLs originating from countries outside the Netherlands into the Dutch food chain. In the following sections the different livestock sectors will be briefly discussed

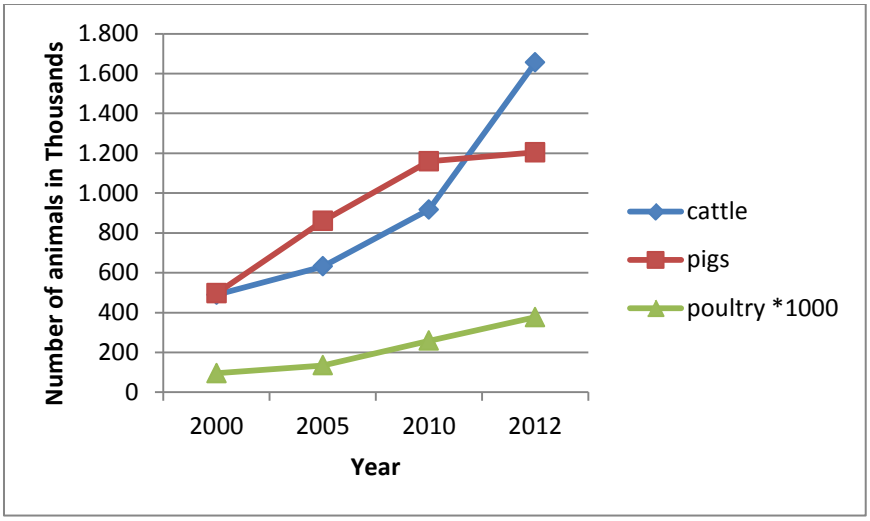


Figure 3.2 *Number of imported animals * 1000 in the Netherlands*

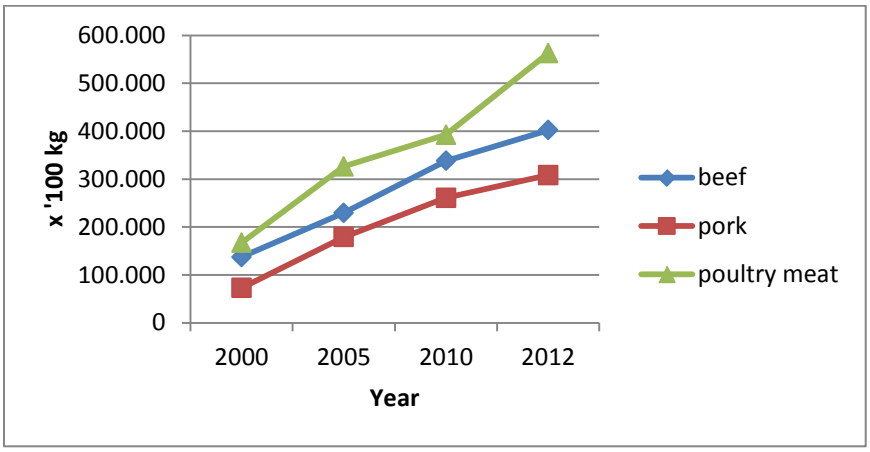


Figure 3.3 *Imported meat in the Netherlands in * 1000 kg.*

As figure 3.2 and 3.3 show the number of live animals as well as meat imported in the Netherlands has increased substantially over the last decade. For pigs and poultry these are mainly slaughter animals for cattle these are partly slaughter animals but also to a large extent calves used for veal calf production.

Cattle

Cattle production is divided in dairy and beef. Trade of live animals occurs for all categories of animals but is likely to be most pronounced for male calves from dairy farms which are sold to veal production (2011). According to the report of Hoste *et al*, 915.000 live cattle were imported into the Netherlands in 2011, of which 892.000 veal calves (Hoste *et al.*, 2013). Table 3.1 gives an overview of the origin of these calves.

Table 3.1

Number of veal calves imported into the Netherlands in the period 2005-2012

	2005	2010	2012
Germany	283,565	399,059	435,407
Poland	163,327	140,673	115,327
Belgium	68,331	99,864	97,474
Ireland	42,732	66,663	16,146
Lithonia	29,358	63,557	45,015
Denmark	13,933	16,071	30,799
Italy	13,630	4,501	13,229
Luxemburg	9,804	7,088	6,264
Slovakia	6,413	11,157	12,714
Czech Republic	5,154	20,430	30,864
Latvia	1,639	2,882	36,065
Estonia	899	24,283	23,776
Romania	0	10,585	2,017
other	3,748	2,059	379
Total	638,785	866,813	865,097

Although Germany, Poland and Belgium supply the largest part of these calves a whole array of other countries supply these calves. Veal calves at Dutch farms often have multiple origins from different farms in different countries. This creates a large risk of introduction and spread of ESBLs within such veal farms (further facilitated by the frequent use of antibiotics in these farms).

The following graph gives an indication of volumes of meat imported as well as the most important origins.

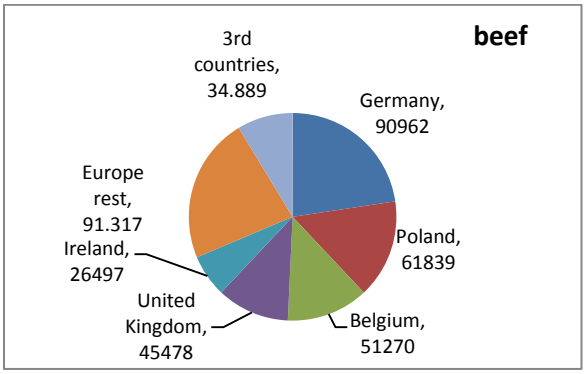


Figure 3.4 *Origin of beef imported into the Netherlands in 2012 (Data source: Comtrade HS data)*

Pigs

Most transports between EU countries of live pigs are between neighbouring countries. Germany is the major importer, Denmark the major exporter of live pigs. The Netherlands is a substantial exporter of finishing pigs for slaughter (2011). According to Hoste *et al* 1.016.000 live pigs were imported into the Netherlands in 2011, of which 980.000 fattening pigs (Hoste *et al.*, 2013). Although the largest part of

the animals comes from Belgium and Germany (cross border) substantial numbers of animals come from all over Europe. As shown in figure 3.5, imported live pigs and pork have substantial differences in origin.

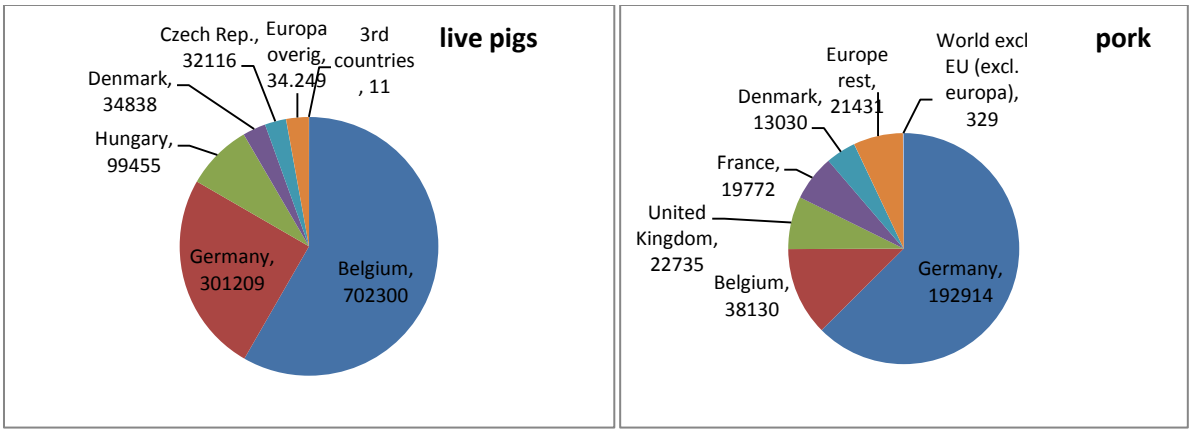


Figure 3.5 *Origin of imported pigs and pork in the Netherlands 2012 (Data source: Comtrade HS data)*

Broilers and poultry meat

The main flow of trade of fattening broilers is between the three neighbouring countries Belgium, the Netherlands and Germany, whereas France is biggest in slaughter of chicken. The main transport routes for breeding poultry are from the Netherlands to Germany, from Czech Republic to Slovakia and Poland and from France to Spain. The trade can be either day-old chicks for broiler farms or day-old chicks of parent- or grandparent stock (2011).

In figure 3.6 it is shown that Germany is the major supplier of live broilers to be slaughtered in the Netherlands and poultry meat is coming from all over the world.

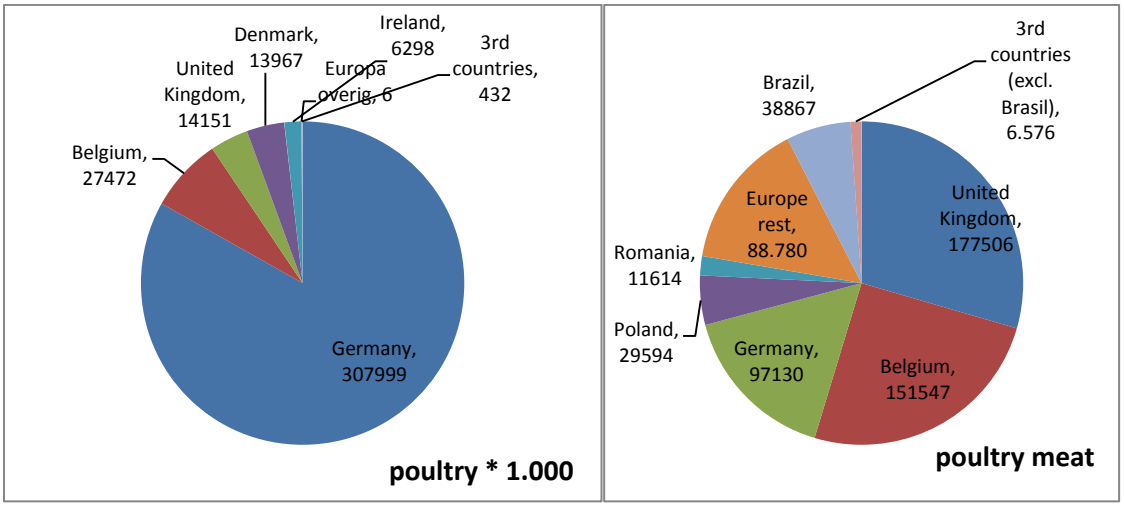


Figure 3.6 *Origin of imported poultry and poultry meat in the Netherlands in 2012 (Data source: Comtrade HS data*

The EFSA report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2012, indicate (except for Brazil and the UK on which no reports exists) similar or higher ESBL status in the different animal species within our main import countries (Germany, Belgium and Poland) compared to the Netherlands (see also Table 1.1; paragraph 1.2.3.1)(2014). This is based on non-selective culturing of commensal *E. coli* bacteria. Whether these data is representative for the ESBL-status of imported animals is not known.

Due to facilitating international trade as a result of implementing SPS regulation and WTO trade agreements in the Netherlands, the import of live animals and the import of meat have more than tripled

in the last decade. Our direct neighbours Germany and Belgium are the main suppliers of live animals to be slaughtered in the Netherlands; however imported animals can originate from all over the EU. This especially is the case for veal calves where trade patterns can change rather quickly over successive years.
Meat trade is even less restricted by distance. The processing industry is supplied by meat from all over the world. All these imports contain the risk of introducing ESBLs.
Although a systematic review of the contribution of imports to the occurrence of ESBLs in the Netherlands is needed to get a detailed insight, initial results indicate that imported animals and meat can contain ESBL producing bacteria (see also paragraph 1.2.3). Their importance is likely to increase when the situation in the Netherlands shows a substantial improvement.

Import of ESBL contaminated live animals or ESBL contaminated meat products, are transmission routes of ESBLs into the Netherlands. Most important countries for import are: Germany (veal calves, beef, live pigs, pork, live poultry, poultry meat), Poland (veal calves and beef), Belgium (live pigs, pork, poultry meat and to a lesser extend live poultry), the UK (poultry meat) and Brazil (poultry meat). The EFSA report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2012, indicate (except for Brazil and the UK on which no reports exists) similar or higher ESBL status in the different animal species in Germany, Belgium and Poland compared to the Netherlands. This is based on non-selective culturing of commensal *E. coli* bacteria. Whether these data is representative for the ESBL-status of imported animals and how import of live animals influence ESBL status within the particular animal sectors is unknown. Therefore the attribution of imports to the Dutch ESBL-situation is unknown. Contamination rates and characteristics of ESBLs in imported meat are currently unknown.

3.9 References

2011, EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on the public health risks of bacterial strains producing extended-spectrum beta-lactamases and/or AmpC beta-lactamases in food and food-producing animals. EFSA Journal 9(8), 1-95.

2014, The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2012 EFSA Journal 12(3).

Aarnink, A.J.A., Roelofs, M., Ellen, H., Gunnink, H., 1999. Dust sources in animal houses. In: International Symposium on Dust Control in Animal Production Facilities, 30 May-2 June, Aarhus (D), p. 34-40 Department(s) Institute of Agricultural and Environmental Engineering Aarhus (D), pp. 34-40.

Appleby, M.C., Hughes, B.O., 1991, Welfare of laying hens in cages and alternative systems: Environmental, physical and behavioural aspects. World's Poultry Science Journal 47, 109-128.

Bakutis, B., Monstviliene, E., Januskeviciene, G., 2004, Analyses of Airborne Contamination with Bacteria, Endotoxins and Dust in Livestock Barns and Poultry Houses. ACTA VET. BRNO 73, 283-289.

Berendsen, B.J.A., Wegh, R.S., Memelink, J., Zuidema, T., Stolker, L.A.M., 2015, The analysis of animal faeces as a tool to monitor antibiotic usage. Talanta 132, 258-268.

Bode, K., Baier, S., Blaha, T., 2007. Specific cleaning and disinfection procedures for Salmonella infected pig herds. In: 13th International Congress in Animal Hygiene (ISAH), Tartu, Estonia, pp. 500-506.

Bokma, M.H., Bergevoet, R., Kemp, B., Rebel, J. 2014. Speerpunten voor samenwerking in onderzoek met betrekking tot antibioticagebruik in de veehouderij., Intern Rapport 201402 Wageningen UR, A.S.G.O.r.i.a.h.e.w.n., ed.

Botteldoorn, N., Heyndrickx, M., Rijpens, N., Grijspeerdt, K., Herman, L., 2003, Salmonella on pig carcasses: positive pigs and cross contamination in the slaughterhouse. Journal of applied microbiology 95, 891-903.

Dierikx, C., Lourens, S., vd Goot, J., Opdam, J., Essen-Zandbergen, A., Fabri, T., Veldman, K., Mevius, D., 2014, Hygienic intervention to diminish cefotaxime resistant *E. coli* bacteria in broilers. in prep.

Dierikx, C., van der Goot, J., Fabri, T., van Essen-Zandbergen, A., Smith, H., Mevius, D., 2013a, Extended-spectrum-beta-lactamase- and AmpC-beta-lactamase-producing Escherichia coli in Dutch broilers and broiler farmers. The Journal of antimicrobial chemotherapy 68, 60-67.

Dierikx, C.M., van der Goot, J.A., Smith, H.E., Kant, A., Mevius, D.J., 2013b, Presence of ESBL/AmpC-producing Escherichia coli in the broiler production pyramid: a descriptive study. PloS one 8, e79005.

Gullberg, E., Cao, S., Berg, O.G., Ilback, C., Sandegren, L., Hughes, D., Andersson, D.I., 2011, Selection of resistant bacteria at very low antibiotic concentrations. PLoS pathogens 7, e1002158.

Hiroi, M., Matsui, S., Kubo, R., Iida, N., Noda, Y., Kanda, T., Sugiyama, K., Hara-Kudo, Y., Ohashi, N., 2012, Factors for occurrence of extended-spectrum beta-lactamase-producing Escherichia coli in broilers. The Journal of veterinary medical science / the Japanese Society of Veterinary Science 74, 1635-1637.

Hordijk, J., Mevius, D.J., Kant, A., Bos, M.E., Graveland, H., Bosman, A.B., Hartskeerl, C.M., Heederik, D.J., Wagenaar, J.A., 2013, Within-farm dynamics of ESBL/AmpC-producing Escherichia coli in veal calves: a longitudinal approach. The Journal of antimicrobial chemotherapy 68, 2468-2476.

Horton, R.A., Randall, L.P., Snary, E.L., Cockrem, H., Lotz, S., Wearing, H., Duncan, D., Rabie, A., McLaren, I., Watson, E., La Ragione, R.M., Coldham, N.G., 2011, Fecal carriage and shedding density of CTX-M extended-spectrum {beta}-lactamase-producing escherichia coli in cattle, chickens, and pigs: implications for environmental contamination and food production. Applied and environmental microbiology 77, 3715-3719.

Hoste, H.S., Bolhuis, J., Wisman, J.H. 2013. Economische betekenis en perspectief van de Nederlandse veehandel (LEI), p. 23.

Hurd, H.S., Gailey, J.K., McKean, J.D., Rostagno, M.H., 2001, Rapid infection in market-weight swine following exposure to a Salmonella typhimurium-contaminated environment. American journal of veterinary research 62, 1194-1197.

Lamers, R.A.N., van Baak, M., Jehoel, G. 2012. Controle antibiotica residuen in drinkwatersystemen pluimveebedrijven (Productschappen Vee, Vlees en Eieren).

Lassok, B., Tenhagen, B.A., 2013, From pig to pork: methicillin-resistant Staphylococcus aureus in the pork production chain. Journal of food protection 76, 1095-1108.

Laube, H., Friese, A., von Salviati, C., Guerra, B., Kasbohrer, A., Kreienbrock, L., Roesler, U., 2013, Longitudinal monitoring of extended-spectrum-beta-lactamase/AmpC-producing Escherichia coli at German broiler chicken fattening farms. Applied and environmental microbiology 79, 4815-4820.

Letellier, A., Messier, S., Pare, J., Menard, J., Quessy, S., 1999, Distribution of Salmonella in swine herds in Quebec. Vet Microbiol 67, 299-306.

Lo Fo Wong, D.M.A., Hald, T., J., v.d.W.P., Swanenburg, M., 2002, Epidemiology and control measures for Salmonella in pigs and pork. Livestock Production Science 76, 215-222.

Lu, J., Sanchez, S., Hofacre, C., Maurer, J.J., Harmon, B.G., Lee, M.D., 2003, Evaluation of broiler litter with reference to the microbial composition as assessed by using 16S rRNA and functional gene markers. Applied and environmental microbiology 69, 901-908.

Madelin, T.M., Wathes, C.M., 1989, Air hygiene in a broiler house: comparison of deep litter with raised netting floors. British poultry science 30, 23-37.

Mannion, C., Egan, J., Lynch, B.P., Fanning, S., Leonard, N., 2008, An investigation into the efficacy of washing trucks following the transportation of pigs--a salmonella perspective. Foodborne pathogens and disease 5, 261-271.

MARAN 2011. MARAN 2009. Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2009, Central Veterinary Institute (CVI) of Wageningen UR, Lelystad. www.cvi.wur.nl/NL/publicaties/rapporten/maranrapportage/default.html.

Martin, S.A., McCann, M.A., Waltman, W.D., 1998, Microbiological survey of Georgia poultry litter. The Journal of Applied Poultry Research 7, 90-98.

Mather, A.E., Innocent, G.T., McEwen, S.A., Reilly, W.J., Taylor, D.J., Steele, W.B., Gunn, G.J., Ternent, H.E., Reid, S.W., Mellor, D.J., 2007, Risk factors for hide contamination of Scottish cattle at slaughter with Escherichia coli O157. Preventive veterinary medicine 80, 257-270.

Michels, I., Chowdhurry, F., Friese, A., Rösler, U., Freitag, M., Grote, M., 2013. Incorporation of antimicrobial agents into vegetable from manured soil and microbiological effects. In: Workshop 'Pharmaceuticals in soils, sludge and slurry' of the German Federal Environment Agency (18th - 19th June 2013). http://www.umweltbundesamt.de/sites/default/files/medien/376/dokumente/michels_posterabstract.pdf.

Namvar, A., Warriner, K., 2006, Application of enterobacterial repetitive intergenic consensus-polymerase chain reaction to trace the fate of generic Escherichia coli within a high capacity pork slaughter line. International journal of food microbiology 108, 155-163.

Nethmap/MARAN 2014. Nethmap/MARAN 2014. Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2013, Central Veterinary Institute (CVI) of Wageningen UR, Lelystad. www.cvi.wur.nl/NL/publicaties/rapporten/maranrapportage/default.html.

Nilsson, O., Borjesson, S., Landen, A., Bengtsson, B., 2014, Vertical transmission of Escherichia coli carrying plasmid-mediated AmpC (pAmpC) through the broiler production pyramid. The Journal of antimicrobial chemotherapy 69, 1497-1500.

Pal, A., Pekhonen, S.O., Yu, L.E., Ray, M.B., 2007, Photocatalytic inativation of gram-positive and gram-negative bacteria using fluorescent light. Journal of Photochemistry and Photobiology A:Chemistry 186, 335-341.

Pell, A.N., 1997, Manure and microbes: public and animal health problem? Journal of dairy science 80, 2673-2681.

Persoons, D., Haesebrouck, F., Smet, A., Herman, L., Heyndrickx, M., Martel, A., Catry, B., Berge, A.C., Butaye, P., Dewulf, J., 2011, Risk factors for ceftiofur resistance in *Escherichia coli* from Belgian broilers. Epidemiol Infect 139, 765-771.

Rajkowski, K.T., Eblen, S., Laubauch, C., 1998, Efficacy of washing and sanitizing trailers used for swine transport in reduction of Salmonella and Escherichia coli. Journal of food protection 61, 31-35.

Reich, F., Atanassova, V., Klein, G., 2013, Extended-spectrum beta-lactamase- and AmpC-producing enterobacteria in healthy broiler chickens, Germany. Emerging infectious diseases 19, 1253-1259.

RIVM 2010. Risk Profile on antimicrobial resistance transmissable from food animals to humans, P.L. Geenen, M.G.J.K., H. Blaak, A.H. Havelaar, A.W. van de Giessen, ed. (RIVM).

Stanger, K.J., Ketheesan, N., Parker, A.J., Coleman, C.J., Lazzaroni, S.M., Fitzpatrick, L.A., 2005, The effect of transportation on the immune status of Bos indicus steers. Journal of animal science 83, 2632-2636.

Swanenburg, M., Urlings, H.A., Keuzenkamp, D.A., Snijders, J.M., 2001a, Salmonella in the lairage of pig slaughterhouses. Journal of food protection 64, 12-16.

Swanenburg, M., Urlings, H.A., Snijders, J.M., Keuzenkamp, D.A., van Knapen, F., 2001b, Salmonella in slaughter pigs: prevalence, serotypes and critical control points during slaughter in two slaughterhouses. International journal of food microbiology 70, 243-254.

Theunissen, H.J., Lemmens-den Toom, N.A., Burggraaf, A., Stolz, E., Michel, M.F., 1993, Influence of temperature and relative humidity on the survival of Chlamydia pneumoniae in aerosols. Applied and environmental microbiology 59, 2589-2593.

Torok, V.A., Hughes, R.J., Ophel-Keller, K., Ali, M., Macalpine, R., 2009, Influence of different litter materials on cecal microbiota colonization in broiler chickens. Poultry science 88, 2474-2481.

Vucemilo, M., Matković, K., Vinković, B., Jakšić, S., Granić, K., Mas, N., 2007, The effect of animal age on air pollutant concentration in a broiler house. Czech Journal of Animal Science 52, 170-174.

Wang, X., Li, G., Xia, X., Yang, B., Xi, M., Meng, J., 2014, Antimicrobial susceptibility and molecular typing of methicillin-resistant staphylococcus aureus in retail foods in Shaanxi, China. Foodborne pathogens and disease 11, 281-286.

Warriss, P.D., Brown, S.N., Edwards, J.E., Anil, M.H., Fordham, D.P., 1992, Time in lairage needed by pigs to recover from the stress of transport. The Veterinary record 131, 194-196.

Zhao, Y., Aarnink, A.J., Cambra-Lopez, M., Fabri, T., 2013, Viral shedding and emission of airborne infectious bursal disease virus from a broiler room. British poultry science 54, 87-95.

Zhao, Y., Aarnink, A.J., Ogink, N.W.M., De Jong, M.C., Groot Koerkamp, P.W.G., 2011, Effectiveness of multi-stage scrubbers on reducing emissions of air pollutants from pig houses. Transactions of the ASABE 54, 285-293.

Zucker, B.A., Trojan, S., Muller, W., 2000, Airborne gram-negative bacterial flora in animal houses. Journal of veterinary medicine. B, Infectious diseases and veterinary public health 47, 37-46.

4. How do resistant bacteria and antibiotics spread to the environment?

4.1 Manure

Antibiotics in manure: spread to the environment
Geofox-Lexmond conducted a literature review concerning the use of antibiotics in intensive livestock husbandry and the transmission of antibiotics and its metabolites through manure to the environment (Geofox-Lexmond, 2009). Their focus was on two frequently used antibiotics: oxytetracyclines and sulfamethoxazole. They state that on average 20 to 90 % of the administered antibiotics are secreted by the manure (original antibiotics or its metabolites). Regarding tetracyclines and sulfonamides, the secretion rates ranges between 40 and 90 %. Within the animal, antibiotics can be metabolized into derivatives that no longer act as antibiotics. However, when these metabolites of antibiotics are secreted in the manure, they are able to transform in the original, active ingredients.
An important potential route of antibiotics and its metabolites to the environment runs through the manure storage and injection of slurry in the soil. The substances can then be absorbed by plants, adsorb to soil particles and/or leach into surface water and groundwater. Through various routes the substances can eventually end up with consumers, and can give rise to health risks.

In the manure, several factors may influence the degradation of antibiotics, such as temperature, acidity, oxygen concentration, the quantity of water and the bacteria content. Which amount of the antibiotics degrades and metabolize depends inter alia on the storage time of the manure and the circumstances under which the manure is stored. A recent report described the presence of a large number of antibiotics found in faeces from swine and calves (Berendsen *et al.*, 2015). Faeces of 17 animals per farm were collected at a slaughterhouse. In total, samples from 20 swine and 20 cattle farms were included. In 55% of the swine, originating from 80% of the swine farms, antibiotics were detected. For the calves in 75% of the faeces samples, originating from 95% of the cattle farms, antibiotics were detected. Oxytetracycline, doxycycline and sulfadiazine were most frequently detected, followed by tetracycline, flumequine, lincomycin and tylosin. Also last-resort antibiotics, like ciprofloxacin and flumequine were detected. Levels ranged from a few µg/kg faeces (all antibiotics) to several mg/kg (oxytetracycline in calf and pig faeces, flumequine in calf faeces and doxycycline and tylosin in pig faeces). No information was given on the antibiotics recently used at the farm, but withdrawal times should have been taken into account. Whether the finding of residues in manure were the result of illegal use of antibiotics within withdrawal time or a delayed excretion due to re-adsorption or contamination at the farm is unknown.

By excretion through manure and urine antibiotics may spread to soil and/or surface water. Here they can accumulate, spread further, deplete and/or metabolize. Many factors can influence the behaviour of antibiotics in the environment. Geofox-Lexmond state that the amount of manure (and thus antibiotics), pH, temperature, amount of water (hydrolysis), the amount of bacteria, the organic carbon-, clay-, silt- and sand-fractions and the amount of minerals affect the environmental behaviour(Geofox-Lexmond, 2009). In addition, photolysis and the oxygen quantity may play a role. Soluble antibiotics and metabolites spread well into the groundwater and surface water/sediment. Manure that directly enters the surface can cause the spread of antibiotics in surface water and sediment. Oxytetracycline adheres well to organic material, especially to clay particles. Sulfonamides attach poorly to soil particles and therefor easier move into groundwater than tetracyclines.

Antibiotics in soil (soil and groundwater) or surface water can present a direct or indirect effect on organisms. Geofox-Lexmond conclude that the limited available field data indicate a very small chance that antibiotics have a direct effect on soil organisms like earthworms and springtails due to the low concentrations (Geofox-Lexmond, 2009). However, they might affect soil bacteria and microbial processes in the soil. They also conclude that direct effects on human health are not likely. Antibiotics have been demonstrated to be present in consumption crops and drinking water but the concentrations

are so low that no direct effects on human health are expected. It is not clear whether these concentrations were related to veterinary and/or human antibiotic use.
There are several reports in which the concentration of antibiotics in the environment (incl. soil, water and manure) is measured. Hamscher indicated maximum concentrations of antibiotics in manure, soil, ground and surface water, plants and farm dust as presented in Table 4.1 (Hamscher, 2009).

Table 4.1
Emission of antibiotics in the environment through manure (Hamscher, 2009).

Location	Found substance	Max. concentration
Pig manure	Tetracyclines, sulfonamides Lincomycin, trimetoprim, tylosin	Several hundred mg/kg < 0.3 mg/kg
Soil	Tetracyclines Sulfonamides	Several hundred µg/kg < 100 µg/kg
Sediment	Oxytetracycline	Several hundred mg/kg
Groundwater	Tetracyclines, sulfonamides	< 0.3 µg/l
Surface water	Tetracyclines, sulfonamides	< 1.4 µg/l
Plants	Tetracyclines, sulfonamides	< 100 µg/kg

Geofox-Lexmond (2009) identify important knowledge gaps regarding antibiotics in manure and spread to the environment, of which: field studies in which the occurrence of residues of antibiotic substances from livestock in soil and groundwater is examined; how many and which metabolites are formed in farm animals, manure and soil; how many oxytetracycline, sulfamethoxazole and resistance genes are degraded in farm animals, manure, soil and groundwater; about antibiotics in manure and the adverse effects on the manure fauna; how will the antibiotics work out exactly on the bacterial communities in the manure and soil and influence soil processes; accumulation of oxytetracycline, sulfamethoxazole and resistance genes in soil and crops; understanding on the role of the soil as possible reservoir (Geofox-Lexmond, 2009).

Resistance genes in soil and environment
The consumption of antibiotics can lead to the development of resistance in micro-organisms. Resistant bacteria and genes are found in plants, surface water and groundwater and even in drinking water. An increase in resistance genes in the past 40 years was observed in Dutch soils that were exposed to animal manure for decades (Knapp *et al.*, 2010). There is however limited information on the human exposure to resistance genes or resistant bacteria via the environment (e.g. groundwater, surface water and drinking water) and its relevance for human health. The human health risks of the occurrence of antibiotic residues and resistant genes in the environment are unknown.

Resistant genes spread through the same emission routes as antibiotics towards groundwater, surface water and drinking water (see Figure 3.1). Tetracycline and sulfonamide resistant genes have been found in manure from veterinary practice, in agricultural soils and in groundwater underlying pig farms. There is evidence that the increasing consumption of veterinary antibiotics leads to changes in the gene reservoirs of the soil. Bacterial communities in soils, pre-exposed to veterinary antibiotics, revealed higher tolerance towards antibiotics than communities that were not pre-exposed (Ter Laak, 2012). Oosterwegel *et al* focussed in small-scale pilot studies on antibiotic resistance, MRSA and ESBL and studied the plausibility of a relationship between manure/fertilization of soils and resistance in the ground (Oosterwegel *et al.*, 2013). They also examined the possibility of transfer of resistance from the (fertilised) soil towards human. The results of their pilot study indicate that it is not likely that fertilization of agricultural soils currently has led to a substantial build-up of ESBL-producing organisms (CTX-M-1 *E. coli*) and MRSA (ST398) into the ground. They consider it also not likely that fertilization of agricultural soils is an important source for the spread of MRSA ST398 and ESBL (type CTX-M-1 *E. coli*) by air. However, due to the small-scale structure of the study the findings should be interpreted cautiously. Especially for the absence of CTX-M-1 producing *E. coli* in soil and air samples. This could be the result of using manure of CTX-M negative farms in the environment. A study performed by Hartmann *et a*l in France confirmed the presence of CTX-M-1 producing *E. coli* in the soils which were fertilized with CTX-M-1 positive manure (Hartmann *et al.*, 2012). Oosterwegel et al plead for research aimed at the systematic identification of pathogens and resistance in manure in order to anticipate on possible adverse developments in Dutch soils (precautionary principle) (Oosterwegel *et al.*, 2013).

Treatment of manure can influence the bacterial composition of the manure. By composting (drying) of manure bacterial counts inside will decrease. In a study published in 2012 composting of poultry manure injected with 10^6ESBL-producing *E. coli* per gram manure led to a more than 5 log decrease in bacterial counts during composting process. After composting (ESBL-producing) *E. coli* counts decreased to less than 10 cfu/gram manure (Duindam *et al.*, 2012). Less than 6% of poultry manure produced in the Netherlands and used in the Netherlands is spread on the land without treatment. About 33% of untreated manure is exported to Germany and Belgium, the rest is mainly burned or dried by composting facilities (Duindam *et al.*, 2012).Whether this is also the case for cattle and swine manure is unknown.

Manure storage and injection of slurry in the soil is a potential transmission route of antibiotics and their metabolites to the environment. There are several studies that describe the concentrations of antibiotic residues in the environment (manure, soil, water, plants). Important knowledge gaps concern the degree and form of degradation of antibiotics residues in animals, manure and soil; the effect of antibiotics residues on environmental bacterial communities and soil processes; the role of soil as reservoir and the risk that it poses to animals and human.
Animal manure can also contribute to spread of resistant bacteria such as ESBLs to the environment. Systematic identification of resistant bacteria in manure in order to anticipate on possible adverse development in soils is lacking. Moreover, there is only limited information on human exposure to resistance genes or resistant bacteria through the environment and its relevance for human health. Composting (drying) can influence bacterial composition of the manure. A risk assessment and evaluation of all types of manure processing/treatments in relation with degradation of antibiotic residues and reduction of (resistant) bacteria is lacking.

4.2 Bio-aerosols

Bio-aerosols are airborne particles that are partly or totally from biological origin (see 4.4 sub *Manure, bio-aerosols and other sources in animal houses)*.
Animals shed microorganisms mainly by means of faecal excretion, which may contain large amounts of micro-organisms (Letellier *et al.*, 1999; Pell, 1997).The distribution of micro-organisms over the different particle sizes of the bio-aerosols largely determines the travel distance of the particle from the source farm and whether or not this particle will be inhaled by humans living in the vicinity of the farm.

A study of Zhao et al. (2011b) showed half-life times in the air at 21–23^{o}C temperature and 80–85% relative humidity of approx. 43 min for *Enterococcus faecalis,* 27 min for *Mycoplasma synoviae*, 21 min for *Escherichia coli,* and 4 min for *Campylobacter jejuni* (Zhao *et al.*, 2011a). Hoeksma et al (2013) studied aerial survival of different microorganisms: *Escherichia coli* (*E.coli*; Gram -), *Enterococcus mundtii* (*E.mundtii;* Gram +), and *Mycoplasma synoviae* (*M.synoviae*; no cell wall) (Hoeksma *et al.*, 2014). They found that the half-life time of bacteria in wet aerosols ranged from 2 min to 28 min and in dry aerosols this was even longer for *E.mundtii*. Hoeksma et al concluded that the tested airborne bacteria, in this experimental setup, survive long enough to be transmitted over a long distance.

Heederik and IJzermans studied the possible effects of intensive-farming on the health of local residents (potential exposure and health problems) (Heederik and IJzermans, 2011). They showed that PM10 dust concentration on most locations around livestock farms increased compared to the urban background concentration (PM10 = particals with a diameter less than 10 µm). However, the increases were more clearly for the microbiological parameters: in areas with a relatively large number of livestock farms or animals in the vicinity increased endotoxin levels in comparison with the urban background level were measured. *Coxiella burnettii* is measured in bio-aerosols at several locations, especially in areas where Q fever occurred in 2008 and 2009. Additionally, in the vicinity of livestock farms and in areas with many farms more often signals are picked up that indicate the presence of MRSA ST398. In this study no measurements are carried out to other resistant bacteria than MRSA ST398. However, the presence of MRSA ST398 DNA in the air around livestock farms can be seen as an indication that other resistant micro-organisms like ESBL-producing bacteria also can be emitted and present in farm surroundings. But until now for ESBL-producing *E. coli* no studies exist in which a correlation is shown between human carriage of ESBL-producing *E. coli* and living near ESBL-positive farms (Huijbers *et al.*, 2013).

Gibbs *et al* evaluated the levels of antibiotic- and multidrug-resistant bacteria in bio-aerosols in the surroundings of a swine confined animal feed operation in Mid-America (Gibbs *et al.*, 2006). They recovered bacterial concentrations with multiple antibiotic resistance or multi drug resistance inside and outside the plant to at least 150 m downwind at higher percentages than upwind. These concentrations were found even after subtherapeutic antibiotics use was discontinued. They conclude that this can pose a potential human health effect for farm workers or people living in close proximity to these plants.

Local conditions may lead to stronger increases in levels of endotoxins/bio-aerosols in the air, for example in case of a concentration of farms in a limited area or farms with high dust emissions due to specific activities or the absence of dust-reducing measures (Heederik and IJzermans, 2011).

In areas with a relatively high density of livestock farms or animals increased endotoxin levels are measured, as well as more often signals are picked up that indicate the presence of MRSA ST398. Local conditions may lead to stronger increases in levels of endotoxins/bio-aerosols in the air, for example in the case of a high farm or animal density in the area or lack of dust-reducing on-farm measures. Specific information on ESBL-producing bacteria in the vicinity of farms is limited.

4.3 Cadaver disposal

In the Netherlands, on-farm disposal of animal carcasses is not allowed. All livestock carcasses are collected and processed at one central rendering facility. Dutch farmers operate in a strict regulatory environment for handling of animal carcasses. These regulations imply frequent transport of carcasses between livestock farms and the rendering facility (Rendac). Carcasses have to be offered at a suitable spot, covered and inaccessible for birds, rodents, cats and dogs. Carcasses weighing more than 40 kg can be stored on-farm for a maximum of 24 hours before transport to the rendering facility. Only carcasses weighing less than 40 kg that are sufficiently cooled (temperature max 10^{o}C) can be stored at the farm for a maximum period of 7 days. These carcasses have to be stored and offered to the rendering company in a barrel (Hoeksma *et al.*, 2009).
Bonnendahl and Järhult stated that transmission routes from dead livestock to birds can be exemplified by the use of “muladares” in Spain- places were carcasses are left for consumption by scavengers (Bonnendahl and Järhult, 2014). In this way both antibiotics and antibiotic resistant bacteria from an intensive livestock industry can be spread to birds and environment. In the Netherlands, this practice is not allowed. Dead grazing animals like cows, goat and sheep may, however, lie for a little while uncovered in the meadow, thus accessible for birds. Complete combustion usually destroys all bacteria and viruses. At the processing temperatures as applied at Rendac all (pathogenic) micro-organisms will be destroyed. Risks for the spread of pathogens and other (resistant) micro-organisms are in particular related with storage and collection of carcasses on farms and transport from farms to the destruction facility. Biosecurity measures are important (Bokma *et al.*, 2009).
Wilkinson reviewed the available information on the biosecurity of mortality composting in poultry and large animals (e.g. mature cattle and pigs) (Wilkinson, 2007). The use of mortality composting as the main method of carcass disposal on mass-scale is probably only suitable for small- to medium-sized carcasses. He states that composting is a well-established pathogen reduction technology, as it is known to control nearly all pathogenic viruses, bacteria, fungi, protozoa (including cysts) and helminth ova to acceptable low levels. Exceptions to this are the endospore-forming bacteria and prions. He mentions multiple mechanisms to be known to be involved in the inactivation of pathogens during composting, such as exposure to heat, microbial antagonism (including antibiotic production and direct parasitism), production of organic acids and ammonia and competition for nutrients. Temperature (temperature and the length of disposure) is considered the most important factor in pathogen inactivation. Wilkonson made no specific remarks on ESBL or antibiotic resistant bacteria.

On-farm carcass disposal methods are not allowed in the Netherlands. In the Dutch rendering process of carcasses all (resistant) micro-organisms will be destroyed. However, storage and collection of carcasses on farms and transport from farms to the destruction facility can pose a risk of spread of (resistant) micro-organisms. Biosecurity measures are important.

4.4 Wild birds and pest animals

Wild birds and rodents

In their review article on antibiotic resistance in wild birds, Bonnendahl and Järhult (2014) state that many bird species are found to carry antibiotic resistant bacteria, even though they have never (continuously) been exposed to antibiotics, ESBL-producing *E.coli* have been isolated from wild birds from all continents of the world except Australia and Antarctica (Bonnendahl and Järhult, 2014). Many factors seem to contribute to the prevalence of antibiotic resistance among wild birds in a certain geographic location. The authors assume that the characteristics of an area are more important than its actual location. Natural preservation state, livestock and human densities, and the remoteness of an area have been postulated as important factors. Aquatic associated species seem especially prone to pick up antibiotic resistance including ESBL-producing strains. There are indications of spread of antibiotic resistance through migration of wild birds and of the transmission between humans and wild birds and vice versa (Bonnendahl and Järhult, 2014).

An earlier review on the frequency and effects of infection with bacterial pathogens in wild birds (Benskin *et al.*, 2009) reveals that Canadian geese are found to carry antibiotic-resistant *E. coli* and use farmland for grazing, thus creating the opportunity for transfer of drug resistance to cattle and other livestock. It is concluded that, although wild animals do not naturally come into contact with antibiotics, they can become infected with resistant bacteria disseminated by wild birds, and act as reservoirs and vectors of resistant bacterial pathogens. According to the authors this might form a risk of encouraging new health problems in wildlife populations to emerge, as well as new reservoirs of zoonotic diseases to be formed. Guenther *et al* consider it unlikely that pathogens isolated from wildlife have acquired resistance through new parallel mutations in the respective genes (Guenther *et al.*, 2012). Horizontal transfer of resistant genes from clinical isolates or the intake of already resistant bacteria from human waste, sewage, and domesticated animal manure might be more probable. Faecal contaminations can be assumed to be the link between settings with a regular or even constant antimicrobial pressure (livestock farming, aquaculture, human and veterinary clinical settings) and the environment, resulting in a constant release of antibiotic-resistant human and animal bacteria into the environment through wastewater or manure. More than 30 animal species have been found shedding ESBL *E.coli* and most of them were birds or rodents. Animals living in urbanized areas are more likely to carry *E. coli* than animals living in remote areas. ESBL-producing *E. coli* have been detected in urban rats (Norway rats and Black and Brown rats). Rats can easily pick up human waste and often interact with human faeces in the sewage system in urban environments and can therefore easily acquire multiresistant bacteria. The types of ESBL genes are basically the same in human, livestock and wildlife, which strengthens the hypothesis that wildlife isolates resemble those found in animal and human patients.

Flies

Usui *et al* isolated and characterized a third-generation cephalosporin-resistant *E. coli* strain from flies and cattle faeces from a cattle barn (Usui *et al.*, 2013). Cephalosporin-resistant *E. coli* strains were isolated from 14.2% of houseflies, 10.3% of false stable flies and 7.5% of cattle faeces. 27 cephalosporin-resistant strains were tested for the presence of antimicrobial resistant genes. Of the 27 samples, 22 isolates from 11 houseflies, 5 false stable flies and 6 cattle faeces samples harboured the $bla_{CTX\text{-}M\text{-}15}$ gene. All the plasmids that harboured this gene were transferable and were members of incompatibility group FIB. These results suggest that transferable plasmids encoding ESBL were prevalent among flies and cattle. As vectors, flies may play an important role in spreading ESBL-producing bacteria from food-producing animals to humans.

Blaak *et al* described the isolation of ESBL-producing *E. coli* from house flies and blow flies caught at two poultry farms. They detected flies with ESBL genotype. These types, as well as six additional types were present in manure and/or rinse water at the same farm (Blaak *et al.*, 2014b).

Many wild birds are found to carry antibiotic resistant bacteria. E.g. Canadian geese are found to carry ESBL-producing *E. coli* and use farmland for grazing, thus creating an opportunity for drug resistance transfer to grazing livestock. Wild animals can act as reservoirs and vectors of resistant bacteria. With respect to rodents, ESBL-producing *E. coli* until now have only been detected in urban rats. As vectors, flies may also be important in spreading of ESBL-producing bacteria from food-producing animals to humans. Biosecurity measures are important.

4.5 Plant waste/water

Diallo *et al* compared the prevalence of pathogenic and extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* in effluents of a municipal wastewater treatment plant (WWTP) receiving wastewater from a slaughterhouse (Diallo *et al.*, 2013). ESBL-producing *E. coli* were mainly detected in city wastewater (1.7%), compared to slaughterhouse wastewater (0.2%), and treated effluent (0.2%). The results showed that pathogenic and/or ESBL producing *E. coli* were mainly detected in human wastewater, and at a lesser extent in animal wastewater. Treatment failed to eliminate these strains which were discharged into the river, and according to Diallo *et al* these strains could then be transmitted to animals and humans via the environment (Diallo *et al.*, 2013).
Blaak *et al* investigated the prevalence and characteristics of ESBL-producing *Escherichia coli* in four Dutch recreational waters and the possible role of nearby waste water treatment plants (WWTP) as contamination source(Blaak *et al.*, 2014a). Isolates from recreational waters were compared with isolates from WWTP effluents, from surface water upstream of the WWTPs, at WWTP discharge points, and in connecting water bodies not influenced by the studied WWTPs. ESBL-producing *E. coli* were detected in all four recreational waters, with an average concentration of 1.3 cfu/100 ml, and in 62% of all samples. In surface waters not influenced by the studied WWTPs, ESBL-producing *E. coli* were detected in similar concentrations, indicating the existence of additional ESBL-*E. coli* contamination sources. Isolates with identical ESBL-genes, phylogenetic background, antibiotic resistance profiles and sequence type were obtained from effluent and different surface water sites in the same watershed, on the same day; occasionally this included isolates from recreational waters. Recreational waters were identified as a potential exposure source of ESBL-producing *E. coli*. WWTPs were shown to contribute to the presence of these bacteria in surface waters, but other (yet unidentified) sources likely co-contribute.
Szczepanowski *et al* also demonstrated that WWTP bacteria are a reservoir for various resistance genes (Szczepanowski *et al.*, 2009) . Moreover, detection of about 64% of the 192 reference resistance genes in bacteria obtained from the WWTP's final effluents in their research indicated that these resistance determinants might be further disseminated in habitats downstream of the sewage plant.
Waste water of primary farms contaminated with animal manure, such as after animal house cleaning, is considered to be animal manure and thus falls under the scope of the manure law (Meststoffenwet). It is collected in the manure pit.

ESBL-producing *E. coli* are mainly detected in human wastewater, and to a much lesser extend in animal waste water from slaughterhouses. Human wastewater treatment plants (WWTP's) are found to contribute to the presence of ESBL-producing *E. coli* in surface waters, but other yet unidentified sources are likely to co-contribute. Via surface water resistant bacteria can then be transmitted to animals and humans.
Waste water of primary farms contaminated with animal manure is defined as animal manure and follows the transmission routes as described under *Manure.*

4.6 Transport vehicles

Rule et al (2008) tested the hypothesis that current methods of transporting food animals from farms to slaughterhouses in the USA may result in pathogen releases and potential exposures of persons in vehicles traveling on the same road (Rule *et al.*, 2008). Air and surface samples were taken from cars driving behind poultry trucks for 17 miles. Air conditioners and fans were turned off and windows fully opened. Background and blank samples were used for quality control. Samples were analysed for susceptible and drug-resistant strains. Their results indicate an increase in the number of total aerobic bacteria including both susceptible and drug-resistant enterococci isolated from air and surface samples. It is concluded that food animal transport in open crates might introduce a novel route of exposure to harmful microorganisms and may disseminate these pathogens into the general environment. Their findings support the need for further exposure characterization and attention to improving methods of food animal transport, especially in highly trafficked regions of high density farming.

There are no systematic data available on the risk of dissemination of ESBL-producing micro-organisms to the environment by transport vehicles.

4.7 Staff/visitors

Paragraph 1.3.1 points out strong indications for transfer of ESBL-carrying plasmids from an animal reservoir to humans via direct contact. Humans can be infected by animal or human sources, and contaminated staff could in theory (re-)introduce a contamination with ESBL-producing bacteria to the animals or animal products. Infected staff could in theory also infect persons within their own social networks. There are no studies known on estimating the relevance of this theoretical transmission route.

Information on the relevance of transmission of ESBL-producing bacteria from farm staff/farm visitors to persons within their social network is lacking.

4.8 References

Benskin, C.M., Wilson, K., Jones, K., Hartley, I.R., 2009, Bacterial pathogens in wild birds: a review of the frequency and effects of infection. Biological reviews of the Cambridge Philosophical Society 84, 349-373.

Berendsen, B.J.A., Wegh, R.S., Memelink, J., Zuidema, T., Stolker, L.A.M., 2015, The analysis of animal faeces as a tool to monitor antibiotic usage. Talanta 132, 258-268.

Blaak, H., de Kruijf, P., Hamidjaja, R.A., van Hoek, A.H., de Roda Husman, A.M., Schets, F.M., 2014a, Prevalence and characteristics of ESBL-producing *E. coli* in Dutch recreational waters influenced by wastewater treatment plants. Vet Microbiol 171, 448-459.

Blaak, H., Hamidjaja, R.A., van Hoek, A.H., de Heer, L., de Roda Husman, A.M., Schets, F.M., 2014b, Detection of extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli on flies at poultry farms. Applied and environmental microbiology 80, 239-246.

Bokma, M.H., Sterrenburg, P., Swanenburg, M., 2009, Voorstudie kansen en risico's van on-farm verwerking van kadavers (focus op varkens). . Rapport 525, Wageningen UR Livestock Research.

Bonnendahl, J., Järhult, J.D., 2014, Antibiotic resistance in wild birds, Review article. . Upsala Journal of Medical Sciences 119, 113-116.

Diallo, A.A., Brugere, H., Kerouredan, M., Dupouy, V., Toutain, P.L., Bousquet-Melou, A., Oswald, E., Bibbal, D., 2013, Persistence and prevalence of pathogenic and extended-spectrum beta-lactamase-producing Escherichia coli in municipal wastewater treatment plant receiving slaughterhouse wastewater. Water research 47, 4719-4729.

Duindam, J.W., Elsinga, G.J., Bijlsma, W., Elsinga, W., Dierikx, C.M., Veldman, K.T., 2012, Eindrapport van onderzoek naar afdoding ESBL in pluimveemest in composterings- en vergistingsinstallaties in Nederland. . E&I Rapportnummer: 20120501.2012. .

Geofox-Lexmond, 2009, Veterinaire antibiotica in de bodem en oppervlaktewater. Geofox-Lexmond BV, Tilburg.

Gibbs, S.G., Green, C.F., Tarwater, P.M., Mota, L.C., Mena, K.D., Scarpino, P.V., 2006, Isolation of antibiotic-resistant bacteria from the air plume downwind of a swine confined or concentrated animal feeding operation. Environmental health perspectives 114, 1032-1037.

Guenther, S., Bethe, A., Fruth, A., Semmler, T., Ulrich, R.G., Wieler, L.H., Ewers, C., 2012, Frequent combination of antimicrobial multiresistance and extraintestinal pathogenicity in Escherichia coli isolates from urban rats (Rattus norvegicus) in Berlin, Germany. PloS one 7, e50331.

Hamscher, G., 2009, Veterinary drugs in the environment: Current knowledge and challenges for the future. Journal of veterinary pharmacology in veterinary science 32 (suppl 1), 24-25.

Hartmann, A., Locatelli, A., Amoureux, L., Depret, G., Jolivet, C., Gueneau, E., Neuwirth, C., 2012, Occurrence of CTX-M Producing Escherichia coli in Soils, Cattle, and Farm Environment in France (Burgundy Region). Frontiers in microbiology 3, 83.

Heederik, D.J.J., IJzermans, C.J., 2011, Mogelijke effecten van intensieve-veehouderij op de gezondheid van omwonenden: onderzoek naar potentiële blootstelling en gezondheidsproblemen. IRAS Universiteit Utrecht, NIVEL, RIVM, 7 juni 2011. .

Hoeksma, P., Aarnink, A.J.A., Ogink, N.W.M. 2014. Effect of temperature and relative humidity on the survival of airborne bacteria, concept report (Wageningen UR Livestock Research).

Hoeksma, P., Lourens, S., Bokma, M.H. 2009. Policy and Research on Animal Carcass disposal in the Netherlands. , University of California at Davis, California, July 21-23 2009. In 3rd International Symposium: Management of Animal Carcasses, Tissue and Related Byproducts Connecting Research, Regulations and Response (University of California at Davis, California, July 21-23 2009).

Huijbers, P.M., de Kraker, M., Graat, E.A., van Hoek, A.H., van Santen, M.G., de Jong, M.C., van Duijkeren, E., de Greeff, S.C., 2013, Prevalence of extended-spectrum beta-lactamase-producing Enterobacteriaceae in humans living in municipalities with high and low broiler density. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 19, E256-259.

Knapp, C.W., Dolfing, J., Ehlert, P.A., Graham, D.W., 2010, Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940. Environmental science & technology 44, 580-587.

Letellier, A., Messier, S., Pare, J., Menard, J., Quessy, S., 1999, Distribution of Salmonella in swine herds in Quebec. Vet Microbiol 67, 299-306.

Oosterwegel, J.L.V., Kloet, R.R., Loeffen, G.J., Schmitt, H., Kluytmans, J.A.J.W., 2013, Antibioticaresistentie, MRSA & ESBL in de bodem. Geofox-Lexmond BV, Tilburg.

Pell, A.N., 1997, Manure and microbes: public and animal health problem? Journal of dairy science 80, 2673-2681.

Rule, A.M., Evans, S.L., Silbergeld, E.K., 2008, Food animal transport: a potential source of community exposures to health hazards from industrial farming (CAFOs). Journal of infection and public health 1, 33-39.

Szczepanowski, R., Linke, B., Krahn, I., Gartemann, K.H., Gutzkow, T., Eichler, W., Puhler, A., Schluter, A., 2009, Detection of 140 clinically relevant antibiotic-resistance genes in the plasmid metagenome of wastewater treatment plant bacteria showing reduced susceptibility to selected antibiotics. Microbiology 155, 2306-2319.

Ter Laak, T., 2012, Mobility of antibiotic resistance genes in the environment and potential threats for drinking water. Watercycle Research Institute, BTO August 2012. Report number BTO 2012.22.

Usui, M., Iwasa, T., Fukuda, A., Sato, T., Okubo, T., Tamura, Y., 2013, The role of flies in spreading the extended-spectrum beta-lactamase gene from cattle. Microbial drug resistance 19, 415-420.

Wilkinson, K.G., 2007, The biosecurity of on-farm mortality composting. Journal of applied microbiology 102, 609-618.

Zhao, Y., Aarnink, A.J., Ogink, N.W.M., De Jong, M.C., Groot Koerkamp, P.W.G., 2011a, Effectiveness of multi-stage scrubbers on reducing emissions of air pollutants from pig houses. Transactions of the ASABE 54, 285-293.

Zhao, Y., Aarnink, A.J.A., Doornenbal, P., Huynh , T.T.T., Groot Koerkamp, P.W.G., Landman, W.J.M., De Jong, M.C.M., 2011b, Investigation on the Efficiencies of Bioaerosol Samplers for Collecting Aerosolized Bacteria Using a Fluorescent Tracer. II: Sampling Efficiency, and Half-life Time. Aerosol Science and Technology 45, 432-442.

5. Measures to reduce spread of resistant bacteria in the food chain and to the environment

5.1 Measures on farm level

As stated in paragraph 1.2.5 antimicrobial use is one of the selecting factors for antimicrobial resistance in bacteria. Minimizing antibiotic treatments and use them effectively (properly dosed and preferably (where possible) administered to individual animals) will lead to less selection pressure and the development of less antimicrobial strains. Examples of this were already mentioned in paragraph 1.2.5 (Dutil *et al.*, 2010; Nethmap/MARAN, 2014).

In addition to decreasing antibiotic use at farms, other measures can be taken to minimize the occurrence and spread of resistant bacteria. Some of them are investigated and published. For broilers, Nuotio *et al* show an effect of the use of competitive exclusion flora in reducing the occurrence of *E. coli* producing ESBLs in the caeca of broilers (Nuotio *et al.*, 2013). This competitive exclusion (CE) product (BROILACT) consists of caecal flora of an adult healthy hen and was given before the broilers were challenged with ESBL/AmpC producing *E. coli*. The hypothesis was, that if other bacteria than ESBL-producers already had colonized the gut, ESBL-producers would have less opportunity to colonize. For three different ESBL/AmpC-producing strains it was concluded that BROILACT reduced the amount of ESBL/AmpC-producing strains in the caecal flora of the treated broilers. This intervention, the use of CE-flora, to reduce the amount of an ESBL-producing strain in faecal samples of broilers in the first week of life was also determined in experiments at the Central Veterinary Institute (yet unpublished data). There it was found that treating the birds with CE-flora before challenging them with ESBL-producing bacteria reduced the amount of ESBL-producing *E. coli* in their faeces. This effect was not found when the birds were first challenged with ESBL-producing *E. coli* and then treated with CE-flora. It seems critical at what point in life broilers are challenged with ESBL producing bacteria and at what time CE flora has been taken up by the birds. Although these experiments give promising results nothing is yet known on the course of the contamination with ESBL-producers after the first week of life. These questions will be covered in a future field study performed by the Central Veterinary Institute. In which broilers will be treated with CE-flora during processing at the hatchery.

Hygienic measures at a farm will ultimately reduce the amount of bacteria at a farm and therefore it will also reduce the amount of resistant bacteria. Several studies support this. Snow et al. (2012) showed that farms that disinfected the calf equipment more frequently than every month had a lower risk of having ESBL *E. coli* present at the farm. Feeding equipment can be contaminated with faeces and regular disinfection will reduce the spread of ESBL *E. coli*. The effect of hygienic measures on ESBL prevalence was also determined in a hatchery and a broiler farm with two poultry houses in the Netherlands (Dierikx *et al.*, 2014). In this study, the effect of hygiene interventions at hatchery and broiler farm level was examined. The results showed that although interventions did not lead to a completely ESBL-free environment at hatchery and on the farm, hygienic measures can result in a decreased prevalence of ESBL producing *Escherichia coli* bacteria in broilers. This effect was even seen six months after the last intervention production round.

Other risk factors at (broiler) farm level that not yet have been investigated are the effect of different hatcheries or different breeds and litter material (found as risk factors for the presence of cefotaxime resistant *E. coli* on broiler farms described by Persoons *et al* (Persoons *et al.*, 2011)). The effect of interventions in this context has not yet been investigated.

A protecting effect for having ESBL on a farm was shown by operating a closed farm policy compared to farms that were open and did not quarantine new cattle (Snow *et al.*, 2012)and farms having more than one animal movement per day per 100 animals compared to farms that had less than one animal movement per day per 100 animals (Reist *et al.*, 2013). From this it can be concluded that minimizing animal movements and introduction of new animals to a farm will help to reduce the spread and occurrence of antimicrobial resistance strains on a farm as well.

Storage of slurry at a farm might also act as a source for ESBL-producing strains, which can be transferred to other places when the faecal wastes or slurry is transported to other places. The storage of

slurry on dairy farms in open compartments compared to closed compartments (a slurry pit compared to a tank) also increased the chance of having ESBL producing isolates at the farm (Snow *et al.*, 2012). Therefore measures to treat the slurry or faecal material that will reduce bacteria inside will help to reduce the spread of ESBL-producers.

In calves resistant bacteria seem to fade during age (Hordijk *et al.*, 2013; Hoyle *et al.*, 2004). However, longitudinal data till slaughter age is not available. Before 2011, resistance levels of commensal *E. coli* derived from faeces of veal calves collected at slaughterhouses were very high and intended to increase, but after 2011, as a result of all the measures taken to reduce antibiotic usage in the whole food-producing sector including veal calves, resistance levels dropped (Nethmap/MARAN, 2014).

Measures that have been investigated, for having an effect on the ESBL-prevalence on farms are: the use of bacterial flora to compete with gut flora (competitive exclusion flora) flora early in life (in broilers) and implementing strict rules of hygiene (broiler hatchery and farm). Other factors that are likely to have an effect on ESBL prevalence are: minimizing animal movements at a farm (preferably all-in, all-out systems, or quarantaine new animals before introduction to the resident animals) and treating faecal waste in a way it will reduce bacterial load before using it on land However, data on the effect these measurements have on ESBL prevalence at farms is lacking.

5.2 Measures feed mills

In theory feed mills could be a source for (resistant) bacteria in feed and in animals. In the past there are examples of bacterial contamination in animal feed (Crump *et al.*, 2002). Implementation of adequate control of feed and feed substances used for contamination with ESBL-producers could be one of the measures to minimize the risk of ESBL spread.

5.3 Measures slaughterhouse/meat processing plants

As stated in paragraph 3.5 ESBL-producing bacteria can be introduced at slaughterhouses by contaminated slaughter animals or, less likely, by slaughterhouse staff and personnel. For *Salmonella* contamination, it is known that improved cleaning and disinfection at slaughterhouse will diminish the occurrence of *Salmonella* in lairage of pigs which will reduce the chance that meat will be contaminated with *Salmonella*(Swanenburg *et al.*, 2001). Slaughterhouse hygiene is found to be a determinative factor for managing carcass contamination with *Salmonella* (Botteldoorn *et al.*, 2003). Similar to *Salmonella* (which also can harbour ESBL or AmpC genes) other bacterial species that could carry ESBL genes are known as common inhabitants of the intestinal tract of animals. Therefore, it is expected (and also found) that they contaminate carcasses during the slaughtering process (Reich *et al.*, 2013). Any measure by which microbial contamination is reduced at slaughter, or during further processing and retailing will also indirectly help to contain the spread of ESBL/AmpC-producing bacteria to humans (2011). These measures could contain optimizing processes for carcass decontamination and avoiding recontamination by effective cleaning and personal hygiene management (Lassok and Tenhagen, 2013). Cooking, pasteurization and other techniques that will kill live bacteria on meat will be effective to reduce bacterial contamination of the meat. However, part of the meat will be sold fresh and without surface decontamination the chance is very high that this meat will contain bacteria on the surface or within the whole product in the case of minced fresh meat.

At slaughterhouses any measure by which microbial contamination is reduced at slaughter, or during further processing and retailing will also indirectly help to contain the spread of ESBL-producing bacteria to humans. This has been confirmed by investigations addressing the effect of hygienic measures on the occurrence of *Salmonella* on carcasses. However quantitative data on contamination of the carcass with ESBL-producers during slaughter and the effect of hygienic measurements on this is currently lacking.

The products of concern for the consumer are products containing meat (or other contaminated fresh products) which did not receive any treatment to reduce bacterial contamination. However, when the meat is properly cooked and the consumer is aware of the possibility of cross-contamination from the uncooked meat to other food via unwashed hands or kitchen equipment the chance of transferring the bacteria from the food to the consumers gut is very low.

When food is properly cooked and the consumer is aware of the possibility of cross-contamination occurring in the kitchen the chance of transferring resistant bacteria from the food to the human gut is very low.

5.5 Transport measures

As shown in paragraph 3.8 transport of animals poses an opportunity for exchange of bacteria between animals. Again cleaning and disinfection of trucks can decrease levels of bacteria present in the truck (Rajkowski *et al.*, 1998)however it is very difficult to get a truck completely clean of bacteria. When hygienic measures are taken it is important to include any tools such as brooms, tools for scraping faeces, boards for moving pigs and transport vehicles as well as areas to which the animals have no direct contact such as ante-rooms for changing clothes and boots and alleys for pig movements (Bode *et al.*, 2007).

Hygienic measures at transport vehicles can reduce (ESBL-producing) bacteria counts, but it is difficult to completely clean the vehicles from ESBL-producing bacteria.

5.6 Measures on carcasses/meat products (end-of-pipe)

Numerous technical possibilities exist to reduce or eliminate the contamination of meat by bacteria or bacterial growth. For example, heating, salting, irradiation, treatment with L -ascorbic acid or hydro-chloride solutions. However these technical possibilities to reduce/eliminate the contamination of meat and meat products are only sparsely implemented. Reasons for these limited implementations are: the meat loses specific characteristics (e.g. heating), taste might be affected, regulatory issues but the most prominent reason is lack of social acceptance of these techniques by consumers.

There are several technical possibilities to reduce or eliminate the contamination of carcasses and/or meat by bacteria or bacterial growth. However, they are only sparsely implemented, due to possible undesired side-effects and as most prominent reason: the lack of societal acceptance of these techniques by consumers. Therefore development of novel, safe and acceptable techniques to reduce and control infective loads at carcasses and/or meat is a priority.

5.7 Import measures

Logistic processing of slaughter animals could be applied to minimize the risk of introduction of ESBL via import of live slaughter animals. This implies strict separation of animals and their (waste) products from domestic animals. For example slaughtering at the end of the day after which the slaughterhouse is cleaned and disinfected (or separated slaughter days for imported animals). Different trucks for imported animals which are not used for domestic animals. To minimize import of live animals that will be raised in the Netherlands, quarantine measures and screening for ESBLs could minimize import of positive animals.

Measures to restrict ESBL introduction and spread via import of animals could be focused at logistic processing for slaughter animals and screening and quarantine measurements for animals that will be raised in the Netherlands. However, these measurements have never been investigated in relation to preventing ESBL import via positive animals.

5.8 References

2011, EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on the public health risks of bacterial strains producing extended-spectrum beta-lactamases and/or AmpC beta-lactamases in food and food-producing animals. EFSA Journal 9(8), 1-95.

Bode, K., Baier, S., Blaha, T., 2007. Specific cleaning and disinfection procedures for Salmonella infected pig herds. In: 13th International Congress in Animal Hygiene (ISAH), Tartu, Estonia, pp. 500-506.

Botteldoorn, N., Heyndrickx, M., Rijpens, N., Grijspeerdt, K., Herman, L., 2003, Salmonella on pig carcasses: positive pigs and cross contamination in the slaughterhouse. Journal of applied microbiology 95, 891-903.

Crump, J.A., Griffin, P.M., Angulo, F.J., 2002, Bacterial contamination of animal feed and its relationship to human foodborne illness. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 35, 859-865.

Dierikx, C., Lourens, S., vd Goot, J., Opdam, J., Essen-Zandbergen, A., Fabri, T., Veldman, K., Mevius, D., 2014, Hygienic intervention to diminish cefotaxime resistant *E. coli* bacteria in broilers. in prep.

Dutil, L., Irwin, R., Finley, R., Ng, L.K., Avery, B., Boerlin, P., Bourgault, A.M., Cole, L., Daignault, D., Desruisseau, A., Demczuk, W., Hoang, L., Horsman, G.B., Ismail, J., Jamieson, F., Maki, A., Pacagnella, A., Pillai, D.R., 2010, Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. Emerging infectious diseases 16, 48-54.

Hordijk, J., Mevius, D.J., Kant, A., Bos, M.E., Graveland, H., Bosman, A.B., Hartskeerl, C.M., Heederik, D.J., Wagenaar, J.A., 2013, Within-farm dynamics of ESBL/AmpC-producing Escherichia coli in veal calves: a longitudinal approach. The Journal of antimicrobial chemotherapy 68, 2468-2476.

Hoyle, D.V., Shaw, D.J., Knight, H.I., Davison, H.C., Pearce, M.C., Low, J.C., Gunn, G.J., Woolhouse, M.E., 2004, Age-related decline in carriage of ampicillin-resistant Escherichia coli in young calves. Applied and environmental microbiology 70, 6927-6930.

Lassok, B., Tenhagen, B.A., 2013, From pig to pork: methicillin-resistant Staphylococcus aureus in the pork production chain. Journal of food protection 76, 1095-1108.

Nethmap/MARAN 2014. Nethmap/MARAN 2014. Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2013, Central Veterinary Institute (CVI) of Wageningen UR, Lelystad. www.cvi.wur.nl/NL/publicaties/rapporten/maranrapportage/default.html.

Nuotio, L., Schneitz, C., Nilsson, O., 2013, Effect of competitive exclusion in reducing the occurrence of Escherichia coli producing extended-spectrum beta-lactamases in the ceca of broiler chicks. Poultry science 92, 250-254.

Persoons, D., Haesebrouck, F., Smet, A., Herman, L., Heyndrickx, M., Martel, A., Catry, B., Berge, A.C., Butaye, P., Dewulf, J., 2011, Risk factors for ceftiofur resistance in *Escherichia coli* from Belgian broilers. Epidemiol Infect 139, 765-771.

Rajkowski, K.T., Eblen, S., Laubauch, C., 1998, Efficacy of washing and sanitizing trailers used for swine transport in reduction of Salmonella and Escherichia coli. Journal of food protection 61, 31-35.

Reich, F., Atanassova, V., Klein, G., 2013, Extended-spectrum beta-lactamase- and AmpC-producing enterobacteria in healthy broiler chickens, Germany. Emerging infectious diseases 19, 1253-1259.

Reist, M., Geser, N., Hachler, H., Scharrer, S., Stephan, R., 2013, ESBL-producing Enterobacteriaceae: occurrence, risk factors for fecal carriage and strain traits in the Swiss slaughter cattle population younger than 2 years sampled at abattoir level. PloS one 8, e71725.

Snow, L.C., Warner, R.G., Cheney, T., Wearing, H., Stokes, M., Harris, K., Teale, C.J., Coldham, N.G., 2012, Risk factors associated with extended spectrum beta-lactamase Escherichia coli (CTX-M) on dairy farms in North West England and North Wales. Preventive veterinary medicine 106, 225-234.

Swanenburg, M., Urlings, H.A., Snijders, J.M., Keuzenkamp, D.A., van Knapen, F., 2001, Salmonella in slaughter pigs: prevalence, serotypes and critical control points during slaughter in two slaughterhouses. International journal of food microbiology 70, 243-254.

6. Summarizing important knowledge gaps and how to address these

Although substantial research has been performed to get insight into spread and epidemiology of ESBLs, there are still a number of important knowledge gaps in relation to the understanding of ESBL behaviour and risks for humans. The major identified gaps (without being exhaustive) are presented below.

- Information on dose effect relations for ESBLs/carbapenemases is mostly lacking. Insight is needed into how many ESBL bacteria are necessary to colonize the human gut. Does the source (animal versus human) from which ESBL bacteria are acquired have an effect on this dose-effect relation? The answer to this question is vital to understand which interventions need to be taken to prevent colonization of the human gut with ESBL/carbapenemase producers. Insight is needed into the type of intervention, the needed efficacy of the intervention and where in the livestock production process to intervene. Determining these dose-effect relations is very difficult to investigate and will probably also differ between individuals. A way to address this would be the development of mathematical models that are able to predict dose effect relations

- The risk of introduction of carbapenemase producing Enterobacteriaceae in livestock production or companion animals is unknown. It is very likely the route of transmission will be through human carriers or human waste. There is a need to develop and implement good monitoring programs in animals. This might reduce the risk of spreading carbapenemase producers.

- What the effect is of residue concentrations of antimicrobials on the selection and evolution of antimicrobial resistance needs to be investigated. Insight is needed into the effect of residues in milk from treated cows or residues of antimicrobials excreted in the farm environment through urine or faeces. At this moment, it is unknown how these residues will influence the occurrence and spread of antimicrobial resistant bacteria in the gut and litter/slurry and whether this is important for the spread of antimicrobial resistant bacteria into the food chain.

- Insight is needed into the direct effect of the reduction of the use of antimicrobials in livestock on the occurrence of resistance found in bacteria derived from livestock. It is unknown whether the reductions in antibiotic use will be enough to control ESBL contamination of animals and food and prevent transmission to humans. Also insight is needed into factors that determine ESBL presence within farms or countries other than antimicrobial use. What is the effect of further reduction of selective antibiotics (all beta-lactams) for livestock production for the occurrence of ESBLs?

- Data on contamination of the carcass with ESBL-producers during slaughter and the effect of hygienic measurements on this is currently lacking. Also information on quantitative data of the attribution of ESBLs in food and animals derived from other countries is lacking. Although a monitoring program on ESBL producing bacteria on meat found in Dutch supermarkets exists, the country of origin is not always known. It is not known how much import from other countries contributes to the Dutch problem. If ESBL levels in Dutch animal production system will decrease, the contribution of import (from animals or meat) to Dutch ESBL is likely to increase.

- Is it possible to adapt livestock production systems to diminish transmission between different stages in production chains?

7. Interventions, control and policy measures

7.1 Socioeconomic framework

Most of the research regarding ESBLs and antimicrobial resistance has been aimed at the mechanisms of (preventing) occurrence and spread. Understanding of the results of this research is pivotal for a successful prevention of spread. Important determinants were antibiotic use and infection control and hygiene on farms and in production chains. It is likely that interventions aiming at reducing or eradicating the prevalence of ESBLs will involve practices to be implemented at farm level.
When it comes to the choice of interventions, there is no 'silver bullet' or 'magic carpet'. Instead, it is widely accepted that combinations of interventions are required to deal with the complexities of many policy issues (Murphey *et al.*, 2012). Several interventions will be necessary to realise the objectives. Probably, different interventions will be needed for different groups of farmers (and other relevant stakeholders), depending on the socio-economic context. Not all (technical) measures are equally suitable to be implemented by all stakeholders, and the effectiveness of the implementation partly depends on stakeholders' socio-economic environment and circumstances.
Farmers together with their veterinarians are important decision makers when new implementing practices that lead to minimizing the risk of occurrence and pollution of the environment with ESBLs. Some common farm practices need to be changed or a specific practice should be stopped. For successful implementation of the knowledge/innovation/research regarding ESBL by stakeholders, attention should be given at those mechanisms that can effectively change the farmers behaviour.
Farmers behaviour can be influenced using various institutional mechanisms: legal instruments, economic rewards, provision of advice and voluntary collective actions (Blackstock *et al.*, 2010). Interventions designed to change farmers behaviour usually include one or a number of the institutional mechanisms. Designing successful interventions requires an appropriate method for characterising interventions and linking them to an analysis of the targeted behaviour.
At the moment no research is available that provides attention into the mechanisms that lead to successful implementation of the existing knowledge/innovation/research regarding ESBL by stakeholders. To get an insight in potential usefulness of these mechanisms we had to turn to the general literature on behavioural change as well as to specific literature on influencing behaviour regarding public goods[2] like water quality. Blackstock et al. (2010) in their literature review concluded that (1) linking advice to behavioural change should be viewed in the context of understanding differences between farmers and (2) that farmers have multiple factors which influence their behaviour, some of which are related to economic wellbeing and production objectives for their enterprise but others are related to their own identity and the influence of peer interaction.
If a strategy for ESBL reduction consists of a specific set of (technical) measures, then the socio-economic conditions should be optimized to stimulate the implementation of these measures. Per measure, the need for interventions could be assessed, based on a survey of the behavioural characteristics and personal preferences of the farmers. This part of the evaluation leads to 'tailor-made' recommendations for implementation, thus probably different for each (group of) farmer(s) on the need for interventions to stimulate the desired behaviour.
Ypma and Van Gaasbeek (2011) define a number of conditions that have to be fulfilled for successful change: increasing pressure to change ('sense of urgency'); clear common objectives; clear relationship between objectives and interventions; capacity to change; and phased implementation (Ypma and van Gaasbeek, 2001).
To evaluate the effectiveness of interventions aimed at reducing of ESBLs levels in livestock in the Netherlands, a multidisciplinary approach is needed (figure 7.1).

[2] A public good is a product that one individual can consume without reducing its availability to another individual and from which no one is excluded. Economists refer to public goods as "non-rivalrous" and "non-excludable". Good water quality, clean air but also freedom of ESBLs could all be considered public goods.

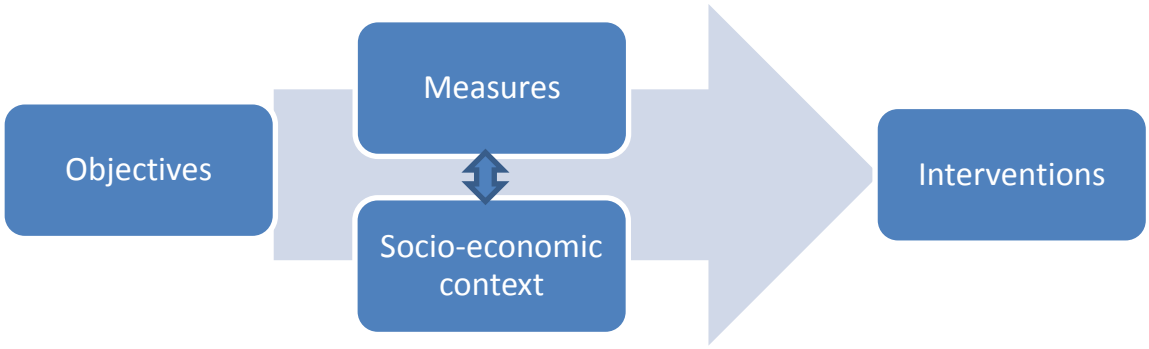


Figure 7.1 *General approach of the ESBL evaluation framework*

A strategy for ESBL reduction might either start with a certain measure, for example the acidification of drinking water for livestock. Subsequently, the socio-economic conditions should be optimized to stimulate the implementation of this measure. An alternative strategy may start with the implementation of an incentive system in the supply chain (e.g., bonus for low prevalence level and/or malus for high prevalence level), which will stimulate farmers to take appropriate action (i.e. implement one or more relevant measures). The likelihood of implementation should be evaluated on a number of socio-economic criteria Table 7.1.

Table 7.1

Socio –economic criteria to consider when implementing technical measures on farm level

socio-economic criteria	**Description**
Cost-effectiveness on farm level	Many studies show that the cost of implementing a measure is one of the crucial factors influencing the actual behaviour of the farmer. Most farmers will only take an expensive measure if the expected revenues will outweigh costs, or if the measure is important for another reason.
Practical feasibility	Besides costs, also the practical feasibility is important. Even a cheap measure will not be taken if it requires time and or effort, that are not available, or if it requires special skills, that the farmer might not have yet. Legislative limitations or licences are other relevant aspects of feasibility.
Relevance at sector level	The impact of applying a specific measure is influenced by the current level of implementation. If it is a measure that most farmers have already implemented, the additional impact on sector level will be relatively small. Nevertheless, the measure in itself may be effective and can be part of an intervention.
Societal impact	The measures to be taken have to be acceptable from a societal point of view. Measures with an expected negative societal impact are not preferable. In terms of human health improvement, the revenues of the measures could be quantified in so called DALYs and QALYs. Furthermore, there could be an improvement of consumer trust and risk perception of livestock production among citizens / consumers.
Undesirable side effects	The preferred measures will need to have little or no negative impact on other important aspects, such as animal welfare or the environment.
Behavioural characteristics of the person/farmer to apply the measures	It is important to get insight into the opinion/attitude of farmers (and other relevant stakeholders) towards possible measures, and to understand why farmers (and other relevant stakeholders) do or do not behave as expected on the basis of purely rational grounds.

Based on the insights obtained from behavioural analysis, interventions can be developed to facilitate the adoption of relevant measures (Breukers *et al.*, 2013; Breukers *et al.*, 2012).
The implementation of (technical) measures in a socio-economic context can best be described as an intentional behavioural change. A theory often used to describe intentional behaviour is the Theory of Planned Behaviour (TPB) (Ajzen, 1991), which can give valuable insights into the personal characteristics of the actors that might influence the behavioural change. This theory states that a person's intention to perform a behaviour is predominantly determined by three determinants:

- Attitude
  The personal favourable or unfavourable evaluation of taking the specific measure: own motivation, expectations and perceived importance of the measure. The motivation will also be influenced by the perceived relationship between the measure and the objective (Ypma and van Gaasbeek, 2001).
- Subjective norm
  The social pressure to take measures, determined by perceived expectations from others and its importance. This determinant is not about legal obligations.
- Perceived behavioural control
  Does the person expect to be able to take the intended measures? The ability includes both self-efficacy (i.e. having the means and skills perceived necessary for performing the behaviour) and controllability (the level to which one experiences full control over his own behaviour).

The theory has been proven to be successful in different domains among which the agricultural domain (Beedell and Rehman, 2000; Bergevoet *et al.*, 2004; Breukers *et al.*, 2012; Colemont and Van den Broucke, 2008; de Lauwere *et al.*, 2012; Fielding *et al.*, 2008). In summary, behavioural intention is determined by the extent to which a person considers oneself willing, pressed, and able to take the intended measures.
The socio-economic evaluation results in a selection of interventions with a high potential contribution to the realisation of the objectives. Promising (technical) measures have for example a broad relevance on sector level, high expected epidemiological effectiveness, low costs and a good practical feasibility. In the case of ESBLs, measures will probably be part of "preventive animal health management", with extra attention to biosecurity measures and restrictive use of antibiotics.
Understanding the reasons for decisions and behaviour amongst stakeholders is critical to mitigate agricultural impact on ESBL occurrence. Farmers will still need to draw on reliable scientific advice from experts. It is clear however, that for some farmers, demand-driven information systems are supplanting supply-driven extension (Garforth *et al.*, 2003). The discussion about the credibility of the source of advice and understanding how different farmers evaluate scientific discoveries calls for a 'new social contract' for science whereby science becomes one of many perspectives involved in problem framing and problem resolution (Lubchencho, 1998). Therefore, if the relationship between farmers and scientific experts is shifting from knowledge transfer to knowledge exchange, there are implications for how science underpinning ESBLs is conceptualised, conducted and communicated (Carolan, 2006).
Incentive mechanisms like bonus or penalties are widely used by policy makers to motivate stakeholders towards wanted behaviour. Trversky's and Kanheman's "prospect theory" changed the view on the human economic actor from a fully rational decision maker in the neo-classical economic theory. The theory states that people make decisions based on the potential value of losses and gains rather than the final outcome, and that people evaluate these losses and gains using certain heuristics. Their theory states that humans put more utility[3] to a loss than to a gain. Therefore people are likely to put more effort in avoiding a loss than receiving a gain. Framing of a situation either as a gain or a loss therefor has an impact on the amount of effort that needs to made by policy makers/principals to change farmers/ agents behaviour (Kahneman and Tversky, 1979). Application of this insight into policy design by making smart incentive systems offers potential. Figure 7.2 illustrates that different limiting factors require different intervention methods. For example, a training course is effective to solve the problem of insufficient skills, but is less effective to improve attitude/motivation.

[3] Utility is the ability of something to satisfy needs or wants. Utility is an important concept in economics and game theory, because it represents satisfaction experienced by the consumer of a good.

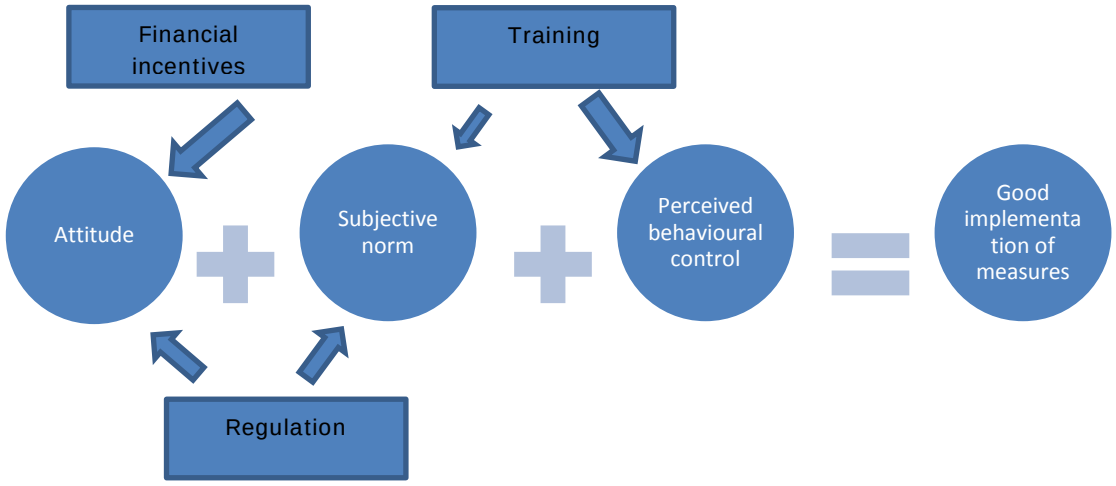


Figure 7.2 *Example of relation between interventions and behavioural characteristics of a farmer to develop adequate interventions, a further analysis of relevant intervention techniques is required.*

7.2 Knowledge gaps on socioeconomic issues and how to address these

- Although the socioeconomic framework and its components are evident on a conceptual level, experience in adequately using this knowledge in the design of intervention strategies is mostly lacking.
- Intervention strategies mostly focus just on one intervention mechanism and therefor most likely targeting only a selection of the population. A more integrated approach of multiple intervention mechanisms is likely to reach a larger audience.
- Motivating farmers on implementing behaviour aiming at maintaining preserving public goods most likely needs a different approach than motivating farmers in cases in which they themselves perceive direct benefits.

To successfully implement interventions aiming at a reduction or elimination of ESBLs besides attention for technical measures, attention should be given to mechanisms that effectively change farmer's behaviour. Cost-effectiveness on farm level, practical feasibility, relevance at sector level, societal impact, absence of undesirable side effects and the behavioural characteristics of the person/farmer all determine the potential success of implementation. Incentive mechanisms like bonus or penalties intelligently used facilitate a successful implementation.

7.3 References

Ajzen, I., 1991, The theory of planned behavior. Organizational Behavior and Human Decision Processes 50, 179-211.

Beedell, J.D.C., Rehman, T., 2000, Using social-psychology models to understand farmers' conservation behaviour. Journal of Rural Studies 16, 117-127.

Bergevoet, R., Ondersteijn, C.J.M., Saatkamp, H.W., van Woerkum, C.M.J., Huirne, R.B.M., 2004, Entrepreneurial behaviour of Dutch dairy farmers under a milk quota system: Goals, objectives and attitudes. Agricultural Systems 80, 1-21.

Blackstock, K.L., Ingram, J., Burton, R., Brown, K.M., Slee, B., 2010, Understanding and influencing behaviour change by farmers to improve water quality. Science of The Total Environment 408, 5631-5638.

Breukers, A., de Wolf, P., Slobbe, R., Molendijk, L., 2013, Duurzaam fytosanitair bodembeheer. Naar een betere beheersing van schadelijke organismen in Nederlandse landbouwgrond. . LEI report 2013-011. The Hague.

Breukers, A., van Asseldonk, M., Bremmer, J., Beekman, V., 2012, Understanding growers' decisions to manage invasive pathogens at the farm level. Phytopathology 102, 609-619.

Carolan, M.S., 2006, Sustainable agriculture, science and the co-production of 'expert' knowledge: The value of interactional expertise. Local Environment 11, 421-431.

Colemont, A., Van den Broucke, S., 2008, Measuring determinants of occupational health related behavior in Flemish farmers: an application of the Theory of Planned Behavior. Journal of safety research 39, 55-64.

de Lauwere, C.C., van Asseldonk, M.A.P.M., van 't Riet, J.P., de Hoop, J.G., ten Pierick, E., 2012, Understanding farmers' decisions with regard to animal welfare: The case of changing to group housing for pregnant sows. Livestock Science 143, 151-161.

Fielding, K.S., Terry, D.J., Masser, B.M., Hogg, M.A., 2008, Integrating social identity theory and the theory of planned behaviour to explain decisions to engage in sustainable agricultural practices. The British journal of social psychology / the British Psychological Society 47, 23-48.

Garforth, C., Angell, B., Archer, J., Green, K., 2003, Fragmentation or creative diversity? Options in the provision of land management advisory services. Land Use Policy 20, 323-333.

Kahneman, D., Tversky, A., 1979, Prospect Theory: An Analysis of Decision under Risk. Econometrica 47, 263-291.

Lubchencho, J., 1998, Entering the century of the environment: a new social contract for science. Science 279 491-497.

Murphey, L., Meijer, F., Visscher, H., 2012, A qualitative evaluation of policy instruments used to improve energy performance of existing private dwellings in the Netherlands. Energy Policy, 1-11.

Ypma, M.E., van Gaasbeek, A.F., 2001, (Im)possibilities of marketing of the environment (“Waar in het bronsgroen eikenhout...; (On)mogelijkheden van vermarkting van de omgeving”). LEI report 7.01.05. The Hague.

8. Summary literature scan

Background on ESBL-producing bacteria

ESBL-producing strains are able to spread vertically by clonal distribution or horizontally by spread of ESBL genes through plasmid conjugation between bacterial species. The type of spread depends on and is related to the type of bacteria, type of ESBL gene and type of plasmid. In the spread of ESBL producing strains between animals and from animals to humans (or vice versa) the mechanism of horizontal spread of plasmids carrying ESBL genes seems most important.

ESBL-producing strains are found in Dutch broilers, pigs, veal calves and dairy cows and meat thereof. The percentage of positive animals is highest in broilers (68%-100%) and lower in pigs, veal calves and dairy cows (57, 46 and 7% respectively). During the last three years ESBL levels in animals in the Netherlands tend to decrease. ESBL-producing bacteria are also found in dogs (45 – 50%), incidentally in cats, in the environment (environmental water), wild birds, and vegetables.

Quantitative info on ESBL presence in different reservoirs is scarce. Information about the minimum dose of ESBL-producing bacteria that can result in colonization of the human gut is lacking. Moreover, the chance of infection in humans that are colonised with ESBL-producing bacteria in their gut is currently unknown.

An important risk factor for the presence of ESBL-producers in animals is the use of antimicrobial agents. Use of cephalosporins and broad spectrum beta-lactams will directly select for ESBL-producing bacteria. Because ESBL-producing bacteria are often multi-drug resistant, also the use of other antibiotics may indirectly have a co-selective effect. This association is well documented in several studies. Besides antibiotic use, other possible risk factors (e.g. transport of contaminated animals or animal products, insufficient hygiene on farms and in production chains, feed and water quality) will differ between animal production sectors. The number of reliable studies to determine other risk factors is still very limited.

Direct contact of people with animals carrying ESBL-producing bacteria increases the chance of colonization of humans. Possible sources of plasmid located ESBL genes for humans are food, but also the environment. The majority of the types of ESBL genes and plasmids found within hospitals are different from those found in food producing animals. This indicates the presence of two distinct ESBL-epidemiology's: one in the hospital and one in food producing animals. A minority of the genes and plasmids that occur in ESBL-producing isolates of humans in Dutch hospitals are genetically associated with genes and plasmids from poultry and poultry products. For other food animal species this genetic association is only demonstrated for genes and plasmids in isolates from farmers and the animals on the farm.

Currently quantitative information on prevalence and characteristics of ESBL-producing bacteria, the genes and plasmids in the open human population are under investigation.

Indirect ESBL transmission from the environment to humans might not play a significant role. However the presence of ESBL-producers in relatively high counts in recreational waters might indicate recreational waters as a potential exposure route to humans. Causal evidence for this is lacking.

Background on carbapenemase-producing bacteria

Until now, reports of the presence of carbapenemase producers are mainly restricted to reports in humans. Carbapenemases in isolates from food producing animals are described in isolated cases of animals in Germany, France and China. However the scarce information might be due to lack of resistance monitoring programs that had included carbapenem antibiotics. Because of the close interaction between pets and their owners and the use of modern broad-spectrum antimicrobials in companion animal health care, a possible introduction of carbapenemase producers in companion animals is more likely than in food-producing animals. However whenever this happens, import of carbapenemase producers in livestock will probably occur via human carriers. Due to their multi-resistant character, it is possible that in spite of the absence of direct selective pressure by use of carbapenems, carbapenemase producers may survive and be transferred in the animal environment. Therefore in 2015, monitoring for carbapenem resistance in livestock will be compulsory in faeces of selected food-animal for the entire EU, including selective isolation of carbapenemase producers. In the Netherlands this program started in 2012 and will be continued on all faecal samples of food-animals collected per year (app. 1500/y).

How and where appear and spread ESBL-producing bacteria in food chains?

Feed mills

Contamination with ESBL-producing bacteria of crops used for animal feed production or used as roughage or bedding material cannot be excluded as a potential source of introduction. Research data are lacking.

Hatcheries (broilers)

There are no studies published yet on contamination rates of hatcheries with ESBLs. Preliminary results show that ESBL-producing bacteria can be present in almost all phases of incubation and manipulation (eggs and chicks) in the hatchery. Dutch hatcheries have stopped using preventive antibiotics.

Primary farms including breeding pyramids

Primary farms have a significant role as amplifiers of resistance. It is demonstrated that ESBL/AmpC producing *E.coli's* are present at all levels of the broiler production pyramid, with the highest levels of contamination at the bottom of the pyramid in the broiler farms. Systematic data on prevalences in the production pyramids of pigs and veal calves is lacking.

The prevalence of ESBL-producing bacteria on primary farms strongly depends on a) the selective pressure by on-farm antibiotic use and b) external and internal biosecurity measures taken to prevent introduction and spread of ESBLs. Information on the negative effects of frequently occurring antibiotic residues in farm environments and the GI-tract of animals is lacking. On farm, contaminated feed pans can become a source of ESBLs spreading during ongoing production periods. Drinking water on pigs and broiler farms have been found to be frequently positive for ESBL-producing *E. coli* and drinking water systems can become an amplifier and distributor of resistance. Insufficient cleaning and disinfection can result in highly contaminated barns and farm environment with ESBL-producing bacteria. Contaminated faeces, litter, bio-aerosols, as well as farm equipment etcetera can transfer and spread ESBLs within barns. Wild/Pest animals can spread all kinds of micro-organisms including ESBL-producing bacteria. Humans can be colonized by animal or human sources. However, colonized humans can also be a source of (re-)introduction of ESBL-producing bacteria, genes, or plasmids in the animals.

Slaughterhouses and meat processing plants

Slaughtering and meat processing are important factors in contamination of animal products with ESBL-producing bacteria. Heat treatments such as scalding and dehairing (e.g. in pig slaughtering) can significantly reduce the ESBL burden on carcasses. However, carcasses can become (re-)contaminated with ESBLs due to contaminated slaughter equipment (as a result of faecal contamination). The contamination rates of the final meat product can also be influenced by handling during meat processing. Effective cleaning and personal hygiene management in slaughterhouses are of main importance to achieve further reduction in ESBL-contamination of animal products, especially in broiler meat production.

Retail

Within retail the highest prevalence of ESBL/AmpC producers is found on poultry meat (83%), with lower prevalences found on turkey meat (35%), beef (5%) and pork (2%). How and where, in which stage of the food production chain, the meat was contaminated is unknown.

Transport vehicles

Not adequately cleaned and disinfected vehicles transporting live animals can be a source of contamination of animals with pathogens like *Salmonella* and *E. coli* due to faecal shedding. Data on contamination of live animals in transport vehicles with ESBLs are lacking. However, optimizing cleaning and disinfection procedures for transport vehicles including tools such as brooms, moving boards and faeces scrapers is considered to reduce the risk of cross contamination of animals with ESBLs.

Overall attribution of imports to NL-situation concerning ESBLs

Import of ESBL contaminated live animals or ESBL contaminated meat products, are transmission routes of ESBLs into the Netherlands. Most important countries for import are: Germany (veal calves, beef, live pigs, pork, live poultry, and poultry meat), Poland (veal calves and beef), Belgium (live pigs, pork, poultry meat and to a lesser extent live poultry), the UK (poultry meat) and Brazil (poultry meat). The EFSA report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2012, indicate (except for Brazil and the UK on which no reports exists) similar or higher ESBL status

in the different animal species in Germany, Belgium and Poland compared to the Netherlands. This is based on non-selective culturing of commensal *E. coli* bacteria. Whether these data is representative for the ESBL-status of imported animals and how import of live animals influence ESBL status within the particular animal sectors is unknown. Therefore the attribution of imports to the Dutch ESBL-situation is unknown. Contamination rates and characteristics of ESBLs in imported meat are currently unknown.

How do resistant bacteria and antibiotics spread to the environment?

Manure

Manure storage and injection of slurry in the soil is a potential transmission route of antibiotics and their metabolites to the environment. There are several studies that describe the concentrations of antibiotic residues in the environment (manure, soil, water, plants). Important knowledge gaps concern the degree and form of degradation of antibiotics residues in animals, manure and soil; the effect of antibiotics residues on environmental bacterial communities and soil processes; the role of soil as reservoir and the risk that it poses to animals and human.

Animal manure can also contribute to spread of resistant bacteria such as ESBLs to the environment. Systematic identification of resistant bacteria in manure in order to anticipate on possible adverse development in soils is lacking. Moreover, there is only limited information on human exposure to resistance genes or resistant bacteria through the environment and its relevance for human health. Composting (drying) can influence bacterial composition of the manure. A risk assessment and evaluation of all types of manure processing/treatments in relation with degradation of antibiotic residues and reduction of (resistant) bacteria is lacking.

Bio-aerosols

In areas with a relatively high density of livestock farms or animals increased endotoxin levels are measured, as well as more often signals are picked up that indicate the presence of MRSA ST398. Local conditions may lead to stronger increases in levels of endotoxins/bio-aerosols in the air, for example in the case of a high farm or animal density in the area or lack of dust-reducing on-farm measures. Specific information on ESBL-producing bacteria in the vicinity of farms is limited.

Cadaver disposal

On-farm carcass disposal methods are not allowed in the Netherlands. In the Dutch rendering process of carcasses all (resistant) micro-organisms will be destroyed. However, storage and collection of carcasses on farms and transport from farms to the destruction facility can pose a risk of spread of (resistant) micro-organisms. Biosecurity measures are important.

Wild animals and pest animals

Many wild birds are found to carry antibiotic resistant bacteria. E.g. Canadian geese are found to carry ESBL-producing *E. coli* and use farmland for grazing, thus creating an opportunity for drug resistance transfer to grazing livestock. Wild animals can act as reservoirs and vectors of resistant bacteria. With respect to rodents, ESBL-producing *E. coli* until now have only been detected in urban rats. As vectors, flies may also be important in spreading of ESBL-producing bacteria from food-producing animals to humans. Biosecurity measures are important.

Wastewater

ESBL-producing *E. coli* are mainly detected in human wastewater, and to a much lesser extent in animal waste water from slaughterhouses. Human wastewater treatment plants (WWTP's) are found to contribute to the presence of ESBL-producing *E. coli* in surface waters, but other yet unidentified sources are likely to co-contribute. Via surface water resistant bacteria can then be transmitted to animals and humans.

Waste water of primary farms contaminated with animal manure is defined as animal manure and follows the transmission routes as described under *Manure*.

Transport vehicles
There are no systematic data available on the risk of dissemination of ESBL-producing micro-organisms to the environment by transport vehicles.

Staff/visitors
Information on the relevance of transmission of ESBL-producing bacteria from farm staff/farm visitors to persons within their social network is lacking.

Measures to reduce spread of resistant bacteria in the food chain to the environment
Measures on farm level
Measures that have been investigated, for having an effect on the ESBL-prevalence on farms are: the use of bacterial flora to compete with gut flora (competitive exclusion flora) early in life (in broilers) and implementing strict rules of hygiene (broiler hatchery and farm). Other factors that are likely to have an effect on ESBL prevalence are: minimizing animal movements at a farm (preferably all-in, all-out systems, or quarantine of new animals before introduction to the resident animals) and treating faecal waste in a way it will reduce bacterial load before using it on land. However, data on the effect these measurements have on ESBL prevalence at farms is lacking.

Measures feed mills
Implementation of adequate control of feed and feed substances used for contamination with ESBL-producers.

Measures slaughterhouse/meat processing plants
At slaughterhouses any measure by which microbial contamination is reduced at slaughter, or during further processing and retailing will also indirectly help to contain the spread of ESBL-producing bacteria to humans. This has been confirmed by investigations addressing the effect of hygienic measures on the occurrence of *Salmonella* on carcasses. However quantitative data on contamination of the carcass with ESBL-producers during slaughter and the effect of hygienic measurements on this is currently lacking.

Measures retail-consumer
When food is properly cooked and the consumer is aware of the possibility of cross-contamination occurring in the kitchen the chance of transferring resistant bacteria from the food to the human gut is very low.

Transport measures
Hygienic measures at transport vehicles can reduce (ESBL-producing) bacteria counts, but it is difficult to completely clean the vehicles from ESBL-producing bacteria.

Measures on carcasses/meat products (end-of-pipe)
There are several technical possibilities to reduce or eliminate the contamination of carcasses and/or meat by bacteria or bacterial growth. However, they are only sparsely implemented, due to possible undesired side-effects and as most prominent reason: the lack of societal acceptance of these techniques by consumers. Therefore development of novel, safe and acceptable techniques to reduce and control infective loads at carcasses and/or meat is a priority.

Import measures
Measures to restrict ESBL introduction and spread via import of animals could be focused at logistic processing for slaughter animals and screening and quarantine measurements for animals that will be raised in the Netherlands. However, these measurements have never been investigated in relation to preventing ESBL import via positive animals.

Interventions, control and policy measures
Socioeconomic framework and knowledge gaps
To successfully implement interventions aiming at a reduction or elimination of ESBLs besides attention for technical measures, attention should be given to mechanisms that effectively change farmer's behaviour. Cost-effectiveness on farm level, practical feasibility, relevance at sector level, societal impact, absence of undesirable side effects and the behavioural characteristics of the person/farmer all

determine the potential success of implementation. Incentive mechanisms like bonus or penalties intelligently used facilitate a successful implementation.

Knowledge gaps on socioeconomic issues and how to address these

Although the socioeconomic framework and its components are evident on a conceptual level, experience in adequately using this knowledge in the design of intervention strategies is mostly lacking. Intervention strategies mostly focus just on one intervention mechanism and therefor most likely targeting only a selection of the population. A more integrated approach of multiple intervention mechanisms is likely to reach a larger audience. Motivating farmers on implementing behaviour aiming at maintaining preserving public goods most likely needs a different approach than motivating farmers in cases in which they themselves perceive direct benefits. However more research is needed the get insight into the exact mechanisms and how this insights lead to effective intervention strategies.

Appendix 2 *Breeding pyramids and chain structures for pig, broiler and veal calves production*

The breeding pyramid structures are specific for pig, broiler and veal calves production. Pig and poultry production consists of a vertically organized production pyramid in which there is a one-direction flow of animals from the top of the pyramid (farms having the nucleus genetic material to final production of animals/eggs for consumption). The number of farms involved in each stage increases moving down the pyramid. This in contrast to the inverse production pyramid in veal calf production in which multiple suppliers supply their calves to one veal calf farm.

Pig production chain
Pig breeding farms keep (nucleus) breeding pigs that supply gilts and/or boars to farrowing farms. Rearing farms provide the rearing of gilts for farrowing farms. Farrowing farms supply pigs to finishing herds. Finishing farms supply slaughter pigs. Pig farms might combine two of the previously mentioned disciplines, i.e., breeding and farrowing or farrowing to finishing.
Figure A2.1 illustrates the structure of the pig breeding pyramid in the Netherlands in 2012.

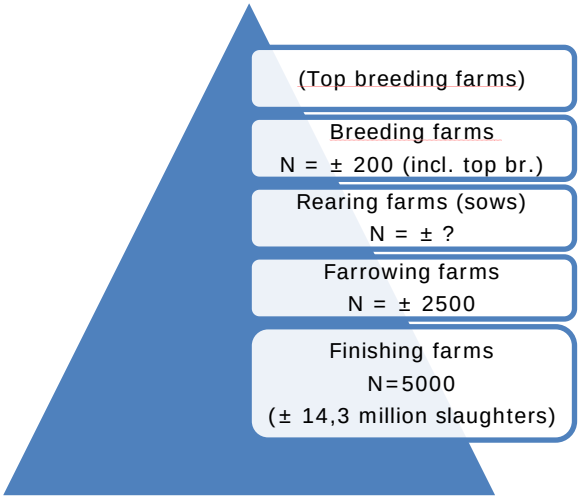


Figure A2.1 *Structure of the pig breeding pyramid in the Netherlands in 2012 (Bron: Productschap Vee, Vlees en Eieren)*

Annually, approximately 900.000 pigs are imported in all stages of the production chain (primary farms, slaughterhouses, processing plants, retail. The major part being slaughter animals going directly to slaughterhouses). On the other hand, 11.4 million pigs are exported and thus leave the Dutch production chain, of which 6.9 million piglets and 4.4 million slaughter pigs (export to Germany resp. 63% and 90%).

Broiler production
The broiler industry has a pyramidal structure in which Pedigree chickens and Great Grandparent Stock (GGPS) on the top through breeding chickens (Grandparent Stock (GPS) and Parent Stock (GP)) produce the broiler chickens on the bottom of the pyramid. In between the stages are the hatcheries, which hatch the eggs and produce the day-old chicks for the next production stage.
In 2012, the Dutch live weight production of broilers amounted almost 998 ktonnes, of which about 43 ktonnes were exported and left the production chain. On the other hand, 185 ktonnes live weight of broilers were imported from abroad and brought into the Dutch production chain. In addition, in 2012 about 407 k tonnes of carcasses and (processed) meat is imported from abroad, whereas about 943 k tonnes of carcasses and (processed) meat is exported. Figure A2.2 illustrates the structure of the broiler breeding pyramid in the Netherlands in 2012.

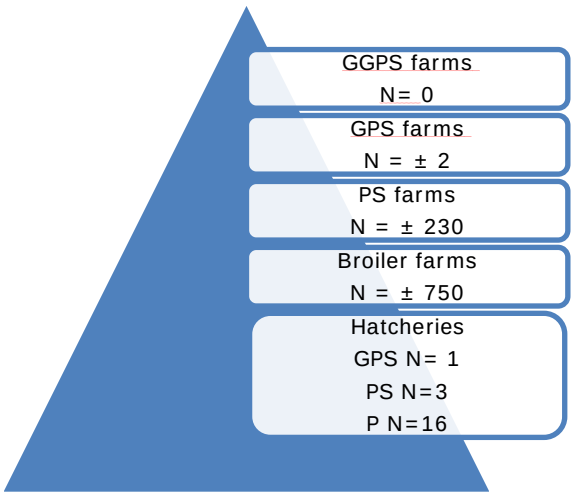


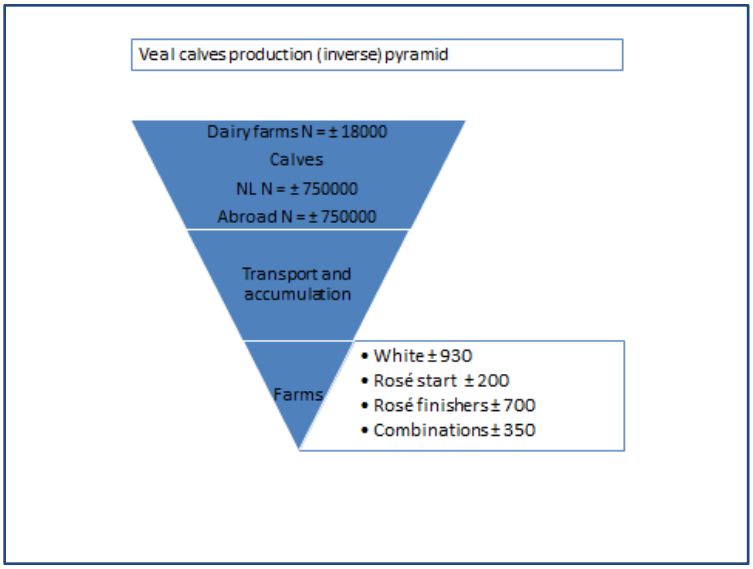
Figure A2.2 *Structure of the broiler breeding pyramid in the Netherlands in 2012 (PPE Koppel Informatie Systeem)*

Veal production

Approximately 1.5 million veal calves are produced yearly in the Netherlands, of which about 50% originate from Dutch dairy farms. The other 50% are imported from abroad, with Germany as main supplier. Important stages in the veal production chain are transport and staging or collection points located abroad or in the Netherlands. There are three different production types of primary farms:

1. White veal farms
2. Rose veal starting farms
3. Rose veal finishing farms

Figure A2.3 illustrates the structure of the veal calves production pyramid in the Netherlands in 2012. It is an inversed pyramid: a large number of farms in the Netherlands and abroad deliver the calves to a relatively small number of veal calf producers.



The export of living calves amounted in 2012 approximately 125.000 (source: PVE; Vee, Vlees en Eieren, Kengetallen 2012, uitgave 2013)

Figure A2.3 *Structure of the veal calves production pyramid in the Netherlands in 2012 (bron: PVE)*

To explore the potential of nature to improve the quality of life

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