Risk assessment and risk management of Extended Spectrum Beta-Lactamases introduction and spread in pigs in agroparks in the Netherlands

Evelien de Olde
870427001
Applied Animal Science
Van Hall Larenstein

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ALTERRA WAGENINGEN UR
University of Applied Sciences
VAN HALL LARENSTEIN
PART OF WAGENINGEN UR

One of the first duties of the physician is to educate the masses not to take medicine.

Sir William Osler, British physician (1849 - 1919)

Table of content

Preface

In the third year of my study, four classmates and I worked on a project for the Animal Health Service on the developments of the use of antibiotics in the pig sector. During this period I was present at a conference where Peter Smeets gave a presentation on Agroparks. I was fascinated by the sense of innovation and the possibilities of this design. After looking into the details, there were several discussions on large scale farming in the Provincial Council of Overijssel. At a meeting of the Provincial Council of Overijssel, on the subject of mega farms, I spoke about the possible solutions Agroparks could offer. Unfortunately the Provincial Council voted against the idea of the Provincial Executive to do a research on the possibilities for Agroparks in Overijssel.

Half a year later I read an article on Agroparks and thought it might be an interesting topic for my thesis. Together with the coordinators of Agropark development at Alterra, Wageningen University, we decided to focus the research specifically on antimicrobial resistance (e.g. MRSA) in pig husbandry in the Netherlands. The work on antimicrobial resistance of Prof. Dr. Dik Mevius, from the Central Veterinary Institute of Wageningen UR, and a publication of the World Health Organization on antimicrobial resistance from food animals, brought the recent concern of ESBL (Extended Spectrum Beta Lactamases) as a high risk factor in animal husbandry under the attention. The combination of these aspects lead to the subject of my research project, and this thesis about risk assessment and risk management of ESBL in pigs in Agroparks in the Netherlands.

I am pleased with the research I did and its results. I would like to thank my supervisors: Madeleine van Mansfeld (Alterra), Peter Smeets (Alterra) and Resie Oude Luttikhuis (Van Hall Larenstein) for their support. I am very thankful for the cooperation of all the experts involved in this research, it has been very motivating to receive emails showing interest in my research and giving positive feedback. The cooperation with international experts made it possible to compare the Dutch selection with the opinions of international experts and made it even more compelling. Next to the participation of researchers, the participation of entrepreneurs from the Dutch pig sector was stimulating, showing their willingness to think jointly about management solutions for this serious problem.

This joint problem solving, which occurred as part of my research project is to me an important step, as I consider ESBL a topic of serious concern which needs further in-depth research and cooperation of different parties. I hope I am able to convince you as an interested reader, to take up this challenge to further this work.

1. Summary

1.1 Introduction

In the Netherlands, the use of antimicrobials is increasing, as well as the level of resistant bacteria in animals. Antimicrobial resistance is defined as the characteristic that a microorganism is less sensitive or insensitive for a medicine. In the total spectrum of antimicrobial drugs, beta lactams, that include antibiotics like penicillin's and cephalosporin's, represent a class of antimicrobials which are important for the treatment of bacterial infections. Resistance to beta lactams has been increasingly observed in bacteria in both humans and animals. (Li et al., 2007) The beta lactam antibiotics can be inactivated by Extended Spectrum Beta Lactamases (ESBL) which are bacterial enzymes. The worldwide emerge of ESBL has been mentioned by the WHO as a matter of particular recent concern. (WHO, 2008)

Human society, animals in agriculture and wildlife and the environment can function as possible reservoirs for ESBL producing bacteria. In the transmission of ESBL's from animals to humans food borne transmission is likely to contribute to the dissemination, since the genes are located on mobile genetic elements and therefore transferable within and between bacterial species. (MARAN 2007)

Agroparks are spatial clusters of different agro-production chains with spatial combination of agro-processing, agro logistics and trade and demonstration. High productive agriculture and processing of agricultural products will take place in a high technological way. Because a combination of both large scale farming and processing is made, ensuring food safety and safety of animal and public health and managing the risks for introduction and spread of ESBLs in Agroparks is necessary. To be able to think of management solutions for the risks, a list of risk factors is needed. Furthermore it is interesting to take a look at how the Agropark Nieuw Gemengd Bedrijf (New Mixed Farm) is going to manage the topic 'antimicrobial resistance' and what additional advice can be given.

1.2. Method

To improve the reliability of the research, a quantitative approach, the conjoint analysis procedure was used to make the research valid and repeatable. To gather a list of risk factors literature on antimicrobial resistance has been reviewed. Fifteen experts from five different countries were involved to add additional risk factors. The experts were then asked to select a maximum of ten risk factors and allocate 100 point to them. This led to three different top 10s of risk factors; one from Dutch experts, from Danish experts and from other international experts. With these selected risk factors and some additional risk factors, the team of Dutch experts were asked to make a final selection during a specially organized work meeting. For this the risk factors were divided into three groups: use of antibiotics, internal bio-security and external bio-security. The experts were again asked to allocate 100 points per group.

With this list of risk factors sixteen experts with different backgrounds (researchers, veterinarians, farmers and people from the Agroparken team of Alterra) were asked to brainstorm on ideas for managing these risks.

1.3. Results

For the use of antimicrobials the most important risk factors are:

1. *Total amount antibiotics per time unit, per animal unit*
2. *Exposure of the intestinal flora to antibiotics*

To maintain internal bio-security the following risk factors are important:

1. *Movement of animals within the farm*
2. *Contact/collective stable with ESBL positive animals now or in the past*
3. *Spread by employees*
4. *Education level of employees*
5. *Thoroughness of terminal hygiene procedures used to clean empty buildings*
6. *Use of collective instruments and materials*.

For maintaining the external bio-security the following risk factors are crucial:

1. *Contact/collective stable with ESBL positive animals now or in the past*
2. *Pigs mixed from different 'sources'*
3. *Transport of animals from different farms to the farm*
4. *Non-use of "all in-all out management" , a continuous throughput of animals in the farm units*
5. *Introduction by people entering the farm*
6. *Education level of employees*

Next to the selected risk factors many different other factors can play a role in the occurrence of ESBL. In the natural environment (soil, water), animals (livestock and wild animals) as well as in the human society ESBL producing bacteria have been found. For that reason maintaining bio-security is important to reduce the possible introduction and spread of ESBL. It is possible to work on these external risks of ESBL however; on the molecular level there are characteristics which are not manageable.

One of the major solutions mentioned during the expert meeting is decreasing the use of antibiotics by improving animal health and the management of the living conditions in the stables. Also hygiene and treatment protocols, good education of employees, design based on bio-security and reduced transport of animals were often mentioned as important measures.

The management of the pig farm of Nieuw Gemengd Bedrijf (NGB) has good ideas on how to manage certain obstacles that today's animal husbandry is facing. When these ideas are implemented, NGB is expected to be able to manage several of the mentioned risk factors and as a consequence have a lower risk for the introduction and spread of ESBL.

1.4. Conclusions

Agroparks can offer new opportunities to manage risk factors for the introduction and spread of ESBL spreading bacteria by their larger scale that reduces per unit costs of biosecurity measures, by their farming system with closed production chains and by their certified quality management.

Field surveys and experimental research will be needed to further elaborate the list of risk factors and their solutions. Because ESBL is observed in many different environments and because of the impact of ESBL on both animal and public health, a multi disciplinary approach for further research is needed. The quantification of expert opinions led to a gathering of knowledge, a list or risk factors, with possible solutions and has highlighted the emergence of ESBL. The results can be used when developing Agroparks and large scale farms to make a step forwards in ensuring food safety and safety of animal and public health, through preventing introduction “at the gate” and spread of ESBL in pigs by more sophisticated animal husbandry practices.

Investments from the government as well as from the sectors are needed to do further research and implement ideas. In this research and development of practical solutions to anticipate on the risk factors, there was the willingness of the different parties involved, to cooperate on this topic and these cooperative efforts should be applied also in the future.

2. Samenvatting

2.1 Introductie

Het gebruik van antibiotica en de mate van antibiotica resistentie in bacteriën neemt toe in dieren in Nederland. Antibiotica resistentie wordt gedefinieerd als de eigenschap van een micro organisme om minder gevoelig of ongevoelig te worden voor een medicijn. In het totale spectrum van antibiotica middelen, beta lactams, zoals penicilline en cefalosporine, zijn een klasse van antibiotica die belangrijk zijn voor de behandeling van bacteriële infecties. Resistentie voor beta lactams wordt in toenemende mate waargenomen in bacteriën in mensen en dieren. (Li et al., 2007) De beta lactam antibiotica kunnen geïnactiveerd worden door de bacteriële enzymen: Extended Spectrum Beta Lactamases (ESBL). Het wereldwijde opduiken van ESBL is door de Wereld Gezondheid Organisatie (WHO) een recente en verontrustende zaak genoemd. (WHO, 2008)

De humane gemeenschap, landbouwhuisdieren, wilde dieren en het milieu kunnen functioneren als mogelijke reservoirs van ESBL producerende bacteriën. In de transmissie van ESBL's van dieren naar mensen, speelt de voedselketen waarschijnlijk een rol in de verspreiding omdat de genen op mobiele genetische elementen liggen die overdraagbaar zijn binnen en tussen bacteriespecies. (MARAN 2007)

Agroparken zijn ruimtelijke clusters van verschillende agro-productie ketens met ruimtelijke combinaties van verwerking, logistiek, handel en demonstratie. Hoog productieve landbouw en verwerking van agrarische producten zal op een hoog technologische manier plaatsvinden. Vanwege de combinatie van zowel grootschalige landbouw en voedsel verwerking, is het garanderen van voedselveiligheid en veiligheid van dier en humane gezondheid noodzakelijk. Het managen van risico's van introductie en verspreiding van ESBL's in Agroparken is daarbij benodigd. Om na te denken over management oplossingen voor deze risico's, is een lijst van risico factoren nodig. Tevens is het interessant om te kijken naar hoe Agropark Nieuw Gemengd Bedrijf het onderwerp 'antibiotica resistentie' gaat managen en welke extra adviezen gegeven kunnen worden.

2.2. Methode

Om de betrouwbaarheid van het onderzoek te verbeteren is gekozen voor een kwantitatieve benadering; de conjoint analysis procedure is gebruikt om het onderzoek beter te gronden en herhaalbaar te maken.

Om een lijst van risico factoren op te stellen is literatuur over antibiotica resistentie geraadpleegd. Vijftien experts uit vijf verschillende landen waren betrokken in het controleren en aanvullen van de lijst van risico factoren. De experts werd gevraag om een selectie van maximaal tien risico factoren te maken en daar 100 punten over te verdelen. Dit heeft geleid tot drie verschillende top 10's van risico factoren; een van Nederlandse experts, van Deense experts en van andere internationale experts. Met deze geselecteerde risico factoren en een aantal extra toegevoegde risico factoren, werd het team van Nederlandse experts gevraagd een uiteindelijke selectie te maken tijdens een georganiseerde werkbijeenkomst. De risico factoren werden hiervoor ingedeeld in drie groepen: gebruik van antibiotica, interne bio-security en externe bio-security. De experts werden opnieuw gevraagd om 100 punten te verdelen.

Met deze lijst van risico factoren werden zestien experts met verschillende achtergronden (onderzoekers, dierenartsen, veehouders en medewerkers van Alterra en het Agroparken team) gevraag om ideeën te brainstormen om de risico's te managen.

2.3. Resultaten

Voor het gebruik van antibiotica zijn de belangrijkste risico factoren:

1. *Totale hoeveelheid antibiotica per tijdseenheid, per dier unit*
2. *Blootstelling van de darmflora aan antibiotica*

Om de interne bio-security in stand te houden zijn de volgende factoren belangrijk:

1. *Verplaatsing van dieren binnen het bedrijf*
2. *Contact/gemeenschappelijke stal met ESBL positieve dieren nu of in het verleden*
3. *Verspreiding door medewerkers*
4. *Educatie niveau van medewerkers*
5. *Zorgvuldigheid van hygiëne procedures gebruikt om lege gebouwen schoon te maken*
6. *Gebruik van gemeenschappelijke instrumenten en materialen.*

Om de externe bio-security in stand te houden zijn de volgende factoren essentieel:

7. *Contact/gemeenschappelijke stal met ESBL positieve dieren nu of in het verleden*
8. *Mengen van varkens van verschillende units of bedrijven*
9. *Transport van dieren van verschillende bedrijven naar het bedrijf*
10. *Geen toepassing van all in - all out management, een continue doorstroom van dieren in bedrijfsunits*
11. *Introductie door mensen die het bedrijf binnenkomen*
12. *Educatie niveau van medewerkers*

Naast de geselecteerde risico factoren spelen vele andere factoren een rol in het voorkomen van ESBL. In het milieu (grond en water), dieren (vee en wilde dieren) en in de humane gemeenschap worden ESBL producerende bacteriën gevonden. Daarom is het belangrijk om bio-security in stand te houden om mogelijke introductie en verspreiding van ESBL te reduceren. Het is mogelijk om te werken aan deze externe risico's van ESBL maar op moleculair niveau zijn er eigenschappen die niet stuurbaar zijn.

Een van de belangrijkste oplossingen die genoemd is tijdens de bijeenkomst is de afname van het gebruik van antibiotica, door middel van verbetering van diergezondheid en het managen van de leefomstandigheden in stallen. Ook hygiëne en behandel protocollen, goede educatie van medewerkers, ontwerpen vanuit het bio-security principe en afname van transport van dieren werden regelmatig genoemd als belangrijke maatregelen.

Het varkensbedrijf van Nieuw Gemengd Bedrijf (NGB) heeft goede ideeën om de obstakels die de hedendaagse veehouderij tegenkomt te managen. Wanneer deze ideeën worden geïmplementeerd, kan worden verwacht dat NGB verschillende van de genoemde risico factoren kan managen en daardoor een lager risico voor introductie en verspreiding van ESBL heeft.

2.4. Conclusies

Agroparken met grootschalige landbouw en een nieuw productie systeem met gesloten productie ketens, bieden nieuwe mogelijkheden om de risico's voor introductie en verspreiding van ESBL positieve bacteriën en andere resistentie pathogenen te managen.

Veldonderzoek en experimenteel onderzoek is nodig om de lijst van risico factoren en de oplossingen te ondersteunen. Omdat ESBL wordt waargenomen in verschillende omgevingen en de impact van ESBL zowel op mens als diergezondheid waarneembaar is, vraagt dit onderwerp een multidisciplinaire aanpak voor toekomstig onderzoek.

Het kwantificeren van expert meningen in dit onderzoek heeft geleid tot een samenkomen van kennis, een lijst van risico factoren met mogelijke oplossingen en heeft het onderwerp ESBL onder de aandacht gebracht. De resultaten kunnen worden gebruikt bij de ontwikkeling van Agroparken en grootschalige veehouderijen om een stap voorwaarts te zetten in het garanderen van voedselveiligheid en veiligheid van dierlijke en humane gezondheid door het voorkomen van introductie "bij de staldeur" en verspreiding van ESBL in varkens in de veehouderij.

Investeringen van de overheid zowel als vanuit de sector zijn nodig om toekomstig onderzoek te doen en mogelijke oplossingen te implementeren. In dit onderzoek was sprake van een sterke bereidheid van de verschillende partijen om samen te werken op dit gebied, deze bereidheid tot samenwerking moet ook in de toekomst worden benut.

3. Introduction

Situation

In the Netherlands, the use of antimicrobials is increasing, as well as the level of resistant bacteria in animals. In the total spectrum of antimicrobial drugs, beta lactams, that include antibiotics like penicillins and cefalosporins, represent a class of antimicrobials which are important for the treatment of bacterial infections. The beta lactam antibiotics can be inactivated by Extended Spectrum Beta Lactamases (ESBL) which are bacterial enzymes. The worldwide emerge of ESBL has been mentioned by the WHO as a matter of particular concern. Also the recently published MARAN report 2007 (Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2006/2007) indicates that next to the frequent occurrence of MRSA in food-animals, of particular concern is the rapid increase in the occurrence of ESBL-producing organism in predominantly poultry and poultry meat products. (MARAN 2007) In the transmission of ESBL's from animals to humans, food borne transmission is likely to contribute to the dissemination, since the genes are located on mobile genetic element and therefore transferable within and between bacterial species. (MARAN 2007)

Antimicrobial resistance is one of the arguments which are used against Agroparks. Agroparks are a new way of agricultural development, with spatial clustering of different agro-production chains with spatial combination of agro-processing, agro logistics and trade and demonstration. High productive agriculture and processing of agricultural products will take place in a high technological way.

Agroparks can be seen as an opportunity to improve issues like animal welfare and animal health. Also ESBL is an important challenge for Agroparks because ensuring food safety and safety of animal and public health is crucial. This can only be done by managing the risks for introduction and spread of ESBLs in Agroparks. To be able to think of management solutions for the risks, a list of risk factors is needed. Furthermore it is interesting to take a look at how the Agropark Nieuw Gemengd Bedrijf (New Mixed Farm) is going to manage the topic 'antimicrobial resistance' and what additional advice can be given. This situation has led to the following research objective and questions:

Research objective

- Risk assessment and risk management of ESBL (Extended Spectrum Beta-Lactamases) introduction and spread in pigs in Agroparks in the Netherlands

Research questions

- What are the risk factors of the introduction and spread of ESBL in pigs?
- How to manage the risks of ESBL introduction and spread of pigs in Agroparks?
- How does the future Agropark Nieuw Gemengd Bedrijf (New Mixed Farm) handle these risk factors of the introduction and spread of ESBL in pigs?

Method

By both literature research and involving experts a list of risk factors and ideas for managing these risk factors can be made. To improve the reliability of the research, a quantitative approach, a so called self explication approach in conjoint analysis, is applied that uses selection and ranking rounds to quantify expert opinions.

4. Literature review

4.1 Antimicrobial resistance and ESBL

Soon after the introduction of antimicrobials like penicillin, resistance to antimicrobials was observed. Antimicrobial resistance is defined as the characteristic that a microorganism is less sensitive or insensitive for a medicine. Resistance is a logical consequence as the resistant bacteria will survive over the susceptible ones, and is an example of Darwin's principle 'survival of the fittest'. (Guardabassi et al., 2008) Antimicrobial resistance is for that reason a serious topic where the medical and veterinary field has to deal with.

Resistance genes and mechanisms existed long before antimicrobials were introduced into medical use and are assumed to originate from in the soil living antibiotic producing bacteria. (Guardabassi et al., 2008) Deep within glaciers in Canada antibiotic resistant bacteria estimated at over 2000 year old have been isolated. (Dancer et al., 1997)

In the total spectrum of antimicrobial drugs, beta lactams represent a class of antimicrobials which are important for the treatment of bacterial infections. Beta lactams antibiotics are used in human and animal health care. Resistance to beta lactams has been increasingly observed in bacteria in both humans and animals. (Li et al., 2007)

Extended spectrum beta lactamases (ESBL) are bacterial enzymes that inactivate essential beta lactam antibiotics like penicillins and cefalosporins (including 3rd and 4th generation cephalosporin's). (Bradford, 2001) The third and fourth generations Cephalosporin's are listed as critically important antimicrobials for human medicine in risk management strategies to contain antimicrobial resistance due to non-human antimicrobial use. (WHO, 2007) ESBL can lead to therapy failure of treatments with this group of antibiotics and is therefore a serious threat. (Pitout and Laupland, 2008)

The prevalence of ESBL was originally related to hospital and nursing facilities but in the last years community-acquired infections have been increasingly observed. (Paterson and Bonomo, 2005) For example CTX-M[1] has spread rapidly in the community during the past years and is the predominant ESBL in many European countries. (Livermore et al., 2007; Cantón and Coque, 2006; Pitout and Laupland, 2008) In the Dutch hospitals an increase of ESBL positive E. coli infections was observed from <1% in 2001 till 4.2% in 2007. (EARSS, 2009) Possible reasons for the increase in the public health care can include the increase of ESBL carriers in the community, hospitalization of patients from nursing homes or from abroad.

Many ESBL genes are located on plasmids which can make it transmittable between bacteria. (Bradford, 2001) Transmissibility appears to be most clinically relevant among members of the family *Enterobacteriaceae* (e.g., *E.coli*, *Salmonella, and Klebsiella*). (Hirsch and Zee, 1999) Liebana et al. indicated in a cattle farm study that horizontal plasmid transfer between strains as well as horizontal gene transfer between plasmids can contribute to the spread of resistance. CTX-M enzymes, a certain group of ESBL, had spread among the *E. coli* intestinal flora of different animals of different age groups. This research indicates the complexity of ESBL and the possibilities of ESBL's to spread. "The close proximity among animals and their

[1] The abbreviation CTX-M is derived from the antibiotic cefotaxime which is hydrolyzed by the CTX-M enzyme

environment and humans makes it possible that genes could be exchanged between microbial populations of different origins." (Liebana et al. 2006)

In the transmission of ESBL's from animals to humans "food borne transmission is likely to contribute to the dissemination, since the genes are located on mobile genetic element and therefore transferable within and between bacterial species." The risks of acquiring ESBLs by humans are different compared to MRSA; MRSA is mainly transmitted by direct contact. (MARAN 2007) The occurrence of MRSA in the Dutch pig sector led to concerns about the public health consequences of large scale farming. Antimicrobial resistance is at the moment used as one of the arguments against Agroparks and large scale farming. It is not possible to say whether the risk for the introduction and spread of antimicrobial resistance is in general higher or lower in Agroparks.

4.2 Risk factors for ESBL

Several factors influencing antimicrobial resistance are mentioned in literature and can be seen as risks for the introduction and spread of ESBL.

The total amount antibiotics used, determines the selection pressure and in that way the selective advantage for ESBL. Strong selection pressure exerted by antimicrobial use, especially with newer generation beta lactam antibiotics, promotes ESBL emergence and subsequent spread. (Kruse et al., 1994) The characteristics, dose, concentration and way of administering the antimicrobial all have their influence on the selection pressure. Just like the environment, the species and population size of the micro organisms present at the infection site and the time period for which the organism is exposed to the antimicrobial. (Guardabassi et al., 2008)

An accurate treatment with antimicrobials should minimize the possible impact of antimicrobial resistance on the efficacy of treatments in the public and animal health. Accurate treatment requires an accurate diagnosis with antimicrobial susceptibility testing, an appropriate antimicrobial agent, administration route and dosage. (Guardabassi et al., 2008) This procedure requires time, money and labor which is limited. An example of a labor saving option is the use of long acting injectable formulations because of the advantage of reducing the need for repeated injections but the disadvantage is that "if products are used that do not reach therapeutic concentration for the pathogens it may lead to treatment failure or encourage resistance development". (Burch et al., 2008) The more modern formulations like Ceftiofur, do achieve sufficient, therapeutic, concentrations over a number of days. This increases there suitability for use and makes them more popular to use and that may result in increased resistance development as well. (Burch, personal comment)

Another labor saving option is treatment with antibiotics is large groups. Of the in 2007 sold antibiotics (590 tonns), 90% was administered through feed or drinking water, meaning it is administered to groups of animals. (Mevius, 2008) The administered antibiotics will work curative for the sick animals and for the others preventive. Different arguments can be found in literature emphasizing the risk of administrating antibiotics through water and feed:

1. Medication by feed and, to lesser degree, water, may result in insufficient uptake by diseased animal due to loss of appetite, thus reducing the effects of medication and increasing the risk of resistance development. (Guardabassi et al., 2008)

2. Soluble products via the drinking water or in liquid feed pass through the stomach more quickly and are therefore more rapidly absorbed than in-feed administration products." (Burch et al., 2008)
3. When systemic treatment is necessary in animal production, intramuscular and intravenous injections are preferable to oral administration to avoid disturbance of the normal gut flora. (Guardabassi et al., 2008)
4. Additional risk associated with oral administration include a risk for farmers to acquire drug traces which might select for resistance when they mix the animal feed themselves and poor hygiene or inhalation could predispose them. (Akwar et al., 2007)
5. The use of in-feed medication in swine rations may lead to increased antimicrobial resistance among fecal *E. coli* in farm residents. *E. coli* in farm residents constitute a potential reservoir for resistant bacteria and/or resistance genes. (Akwar et al., 2007)
6. Finally, exposure of wild animals (including birds and rodents) to animal feed has been mentioned as one of the possible causes of the higher resistance rates in wild animals living close to food animal farms. (Kozak et al, 2009) In that way, wild animals can contribute to the spread of ESBL-producing bacteria when exposed to medicated animal feed.

Next to feed, insufficient hygiene procedures can also be seen as a risk for the spread of ESBL because ESBL positive bacteria are able to survive for months. (Liebana et al., 2006). Exposure of animals to feces directly or indirectly can lead to exchange of bacteria. Also equipment and materials can form a vehicle for ESBL positive bacteria and in that way introduce and spread ESBL positive bacteria in other animal groups. The density of animals per unit or pen is linked to transmission of disease. High animal densities enable fast exchange of bacteria between animals. As the animal is part of a group interactive exchange of bacteria will be possible anyhow, by direct or indirect fecal contact through manure or dust on the farms. (Mevius, 2008)

Mixing animals can lead to introduction of foreign pathogens in animal units. (Kruse et al., 1994) The pigs also have to deal with stress caused by movements and new animals in the group. (Burch et al, 2008) Also a continuous throughput of animals in the stables, not applying all in/all out management can lead to spread of diseases from group to group.

The trucks with animals driving from one farm to another can bring in fecal material with diseases and bacteria. Transport of animals from different farms to the farm, brings together different backgrounds of farms and animals which increases the risk for spread of positive ESBL bacteria. Transport of animals plays an important role in the spread of micro organisms including resistant variants.

People entering the farm are important risk factors as they can carry infectious agents on their skin and clothes. Going through a shower, clothes changing system and a down time without pig contact are mentioned as solutions. (Burch et al., 2008) Persons can introduce or spread ESBL by not taking in account these necessary bio-security measures. The importance of the measures needs to be understood by the farmer and his employees. That is why the education level is also of importance.

Finally, also the environment, including surface water, as a reservoir of ESBL can form a source of disease and a source from which resistance plasmids can easily spread to other pathogens of diverse origins. (Kruse et al., 1994)

4.3 Agroparks and ESBL

ESBL's can form a risk for Agroparks, just like for conventional farms, because transmission of ESBL's from farms to processing chain towards humans can not be left out of consideration. The environment, different animal species and food products can carry ESBL producing bacteria and can function as possible reservoirs. (Kruse et al., 1994; Machado et al., 2008; Jensen et al., 2006; Smet et al., 2008; Girlich et al., 2007) The human society is as well seen as a reservoir in which the food production chain may contribute to the spread of ESBL. (Mesa et al., 2006)

To be able to think of management solutions for the risks, a list of risk factors is needed. Furthermore it is interesting to take a look at how the Agropark Nieuw Gemengd Bedrijf (New Mixed Farm) is going to manage the topic 'antimicrobial resistance' and what additional advice can be given.

When developing Agroparks and large scale farms, managing the risks for introduction and spread of ESBL is necessary to ensure food safety and safety of animal and public health. To manage the risks a list of risk factors and solutions are needed. Literature as well as expert opinions could synthesize a list of risk factors. To improve the reliability of the research, a quantitative approach was used to make the research more valid, repeatable and precise. This method is called the self explication approach in conjoint analysis and applies selection and ranking rounds as methodology to quantify expert opinions. (Green et al., 1990)

Several studies showed that this method is a useful tool in the veterinary field (Horst, 1998; Van Schaik et al., 1998; Van der Fels-Klerx et al., 2000; Noordhuizen et al, 2001). Van der Fels-Klerx et al., for example used this method for a study to quantify expert opinion on risk factors for clinical bovine respiratory disease (BRD) in dairy young stock in the Netherlands and considered the method as a useful complement to field surveys and experimental research. (Van der Fels-Klerx et al., 2000)

5. Method

In the final phase of the study Applied Animal Science, a student has to work on a thesis. When executing an order from a company, tackling a problem and answering research questions should lead to a written report. Mastering a professional procedure, generating knowledge and linking science with day-to-day professional practice are important key words in the thesis.

Because Agroparks as well as a list of risk factors did not yet exist, the best indication of the risks could be reached by expert analysis. A quantification of expert opinion by applying the self explication approach in conjoint analysis was chosen as the methodology to improve the reliability of the research. Dr. Ir. H.J. van der Fels, working for the institute for food quality (Rikilt) has been consulted to discuss the design of this research because she did a study on risk factors for clinical bovine respiratory disease also by quantifying expert opinion.

5.1 Research design

To make a first list of risk factors literature on antimicrobial resistance was consulted. Fifteen experts from five different countries were asked to verify and complete the list of factors affecting ESBL occurrence. Afterwards they were asked to select (maximum) ten most important risk factors from the list of factors and allocate 100 points across the factors to reflect their relative impact on the development and transmission of ESBL.

During the period of desk study the crucial experts to involve in this research were indicated and contacted. Because it is difficult to assess whether the impact of the risk factors scored by the experts is similar to the true impact of the factors in practice, experts with different background (incl. international, medical, food chain and food animal experts) were invited to limit the possible gap between expectations and practice. Experts were defined as people who were working in the field of antimicrobial resistance, epidemiology and/or livestock. They were selected on the base of research or projects they were involved in and on the basis of recommendations of Prof. Dr. D.J. Mevius, professor on antimicrobial resistance at the University of Utrecht and researcher at the Central Veterinary Institute of Wageningen UR.

A test round for evaluating the above described methodology was carried out together with supervisors of Van Hall Larenstein (VHL) and Wageningen University and Research Centre (WUR) Alterra and experts in this methodology. After this, the research method was adjusted, instead of using adaptive conjoint analysis (ACA), a self explication approach in conjoint analysis was used. Arguments for this adjustment include the limited time and necessary requirements for application of ACA.

After a first period of desk study a first list of risk factors for introduction and spread of ESBL was made. Experts were asked to verify the list of risk factors and to add additional risk factors. For the international and Dutch experts this took place by email. In a second email session the experts were asked to make a first selection of risk factors. In Denmark, an expert meeting took place on the 16th of April 2009, with three Danish experts, where risk factors were added and selected. The results were used to compare the selection of other international experts and experts from the Netherlands.

During an expert meeting of six Dutch experts, on the 11th of May 2009, the results of the three top 10's (international, Danish and Dutch) were presented. The experts were asked to select again the most important risk factors and divide 100 point over them. In this selection round, the risk factors were split up into 3 groups, antimicrobial usage, internal bio-security and external bio-security, to make it possible to estimate the relative importance per risk factor.

During this expert meeting, facilitated in a "group decision room" with individual computer facilities, to facilitate selection, ranking, commenting and interactive discussions, the research was presented and discussed. In this round the fourteen invited stakeholders included agropark specialists, farmers, veterinarians, Dutch researchers involved in the morning meeting and other experts related to antimicrobial resistance and farming. (The list of all involved experts can be found in Annex 1.) Important goal in this round was to come with ideas on how to manage the risks in practice, in the development of large farms and Agroparks.

Finally the research will be presented, on the 23rd of June 2009, to the involved stakeholders and others from the agroparken network. This meeting (consult) will be the presentation in which the supervisors will assess the research.

5.2 Data collection

An extended literature study was carried out to get into the different aspects of antimicrobial resistance and ESBL. The risk factors were partly derived from literature and also an input from the email sessions with the experts. Also the expert meeting organized in Copenhagen led to a contribution to the risk factors list. In total the fifteen experts extended the list of risk factors of 50 up to 112 risk factors. Background on the first list of risk factors is given in the literature review. The total list of 112 risk factors is given in annex 3. Afterwards the experts were asked to select a maximum of 10 risk factors. The email sessions and the Danish expert meeting led to three top 10's of risk factors; one for Denmark, one for other international expert and one for the Netherlands.

After this first selection of risk factors it became clear that "the introduction" and "the spread" of ESBL are two different issues which should be selected and ranked differently. In total 55 risk factors were selected by the experts, 9 risk factors were added to this list because they were selected in pre-selections that some experts had sent earlier. The combined list of 64 factors was used during the expert meeting in the Netherlands as the basis for final selection.

During the last expert meeting is appeared that all the risk factors could be combined instead of looking at them from the perspective of "introduction" and "spread". They could be sorted into three different groups;

1. antimicrobial usage
2. internal bio-security
3. external bio-security

The internal bio-security is closely related to spread of ESBL while external bio-security is more about the introduction; the use of antibiotics forms the new group and determines the selection pressure. Together with the experts, the risk factors were divided over the three groups; some risk factors were allocated to more groups. The experts were given the opportunity to think of additional or combinations of risk factors. This led to a number of seven combined risk factors.

Within the three groups selection and ranking took place to be able to identify the main risk factors. The six experts were asked to allocate 100 points over every group of risk factors. The list of risk factors per group is given in annex 4. After the selection and ranking, the experts were asked to write down the argumentation to select this specific risk factor.

After this final selection, ranking and argumentation was given, eight other experts (researchers, farmers, veterinarians, agropark specialists and a representative of the veterinarian pharmaceutical industry) joined the meeting. With the selection and Agropark information as background, a brainstorm on how to manage the risk factors took place.

To specify the term agropark a concrete casus (Nieuw Gemengd Bedrijf) was used on which the risk assessment was carried out. To answer the third research question, an interview with one of the pig farmers was carried out. With the received information the answer on the question: 'How does the future agropark Nieuw Gemengd Bedrijf (NGB) handles these risk factors of the introduction and spread of ESBL in pigs?' can be given.

5.3 Data processing

The input of the experts was processed by grouping, organizing and structuring the answers. A part of the input had to be translated from Dutch to English and needed to be combined as several answers and ideas had overlap. The program Excel was used to make overviews of risks and scores. During the expert meetings in the Netherlands the Group Decision Room (GDR) of Alterra was used. The GDR is a meeting room equipped with an electronic meeting system. It consists of a local computer network of 10 laptops, with software especially developed to support group processes. The meeting room software is used to collect, structure, exchange and preserve information. In the GDR the input of the participants is anonymous, ensuring the input is valued on merit (and less on who has provided the input). Using the GDR also ensures that every participant is allocated the same opportunity to provide input; the participants can react simultaneous. This in contrast to "normal" meetings, where only one person at the time can express his/her opinion. In this way less outspoken persons also have an opportunity to contribute. (Anonymous, 2009) In that way much more can be 'said' in the same time.

6. Results

6.1 Email sessions and expert meeting in Denmark

6.1.1. Gathering risk factors
The first results of this study include the additional risk factors received by email and through the expert meeting in Copenhagen. A first list of risk factors was derived from literature and included 50 risk factors, this was extended by the involved experts to 112 risk factors. The complete list is given in Annex 3. When looking at the entire list of risk factors several main topics can be derived from it, namely; antibiotic use, farm management, people and environment.

6.1.2. First selection and ranking of risk factors
In the second email session the international and Dutch experts were asked to select a maximum of ten risk factors for the introduction and spread of ESBL in pigs and allocate 100 points over the selection. The Danish experts made the selection and allocated points during the expert meeting in Copenhagen. Putting together all the experts per country or per group of countries the following top ten of most important risk factors for the introduction and spread of Extended Spectrum Beta Lactamases can be made.

Denmark
Special for this top 10 is that is has a strong focus on cephalosporin. Due to the fact that the Danish experts are working together for the same institute, the top ten is relatively unanimous compared to the other groups of experts. A high standard deviation of the number 1 and 2 is caused by the selection of number 1, by two experts and the selection of number 2 by the third expert. The first two risk factors are closely related with each other. Based on the minimal and maximal points every risk factor received, it is clear that there is still quite some variation in the points allocated to the different risks. Only the third risk factor received points from all experts.

Only 19 points of the 100 per experts were not allocated to risk factors in the top ten. The numbers 1, 2 and 3 together received more than 50% of the points.

Table 1. Top 10 Denmark

Top 10	Denmark	Mean	Std	Min	Max
1	Use of 3rd and 4th generation cephalosporin's	23.3	20.82	0	40
2	Use of Cephalosporin's in large groups of animals	14.7	25.40	0	44
3	Use of longacting injectable cephalosporin's	13.3	2.89	10	15
4	Age of animal treated with cephalosporin's	8.3	10.41	0	20
5	Use of cephalosporin's in individual animals	5	8.66	0	15
6	Amino penicillin's used	3.3	2.89	0	5
6	Internal hygiene (transmission within the farm)	3.3	2.89	0	5
6	External hygiene (transmission to the farm)	3.3	2.89	0	5
6	Quantities of other antimicrobials used	3.3	2.89	0	5
6	Farmer or farm worker receiving cephalosporin's	3.3	5.77	0	10
	Remaining	**18.9**			

International experts

The results of the international experts are less unanimous, next to the top ten, 20 other factors were selected over which 32 points on average were divided. Special for this group is the occurrence of the risk factor 'exposure to any surface water that may be contaminated with human or animal waste'. This can be due to the fact that not every country has a closed sewage system and manure can be unloaded in ditches. Because of that, surface water can contain animal and human waste with resistant bacteria and exposure to this surface water can lead to exchange of bacteria. All experts gave points to the first risk factors, on the others a large variation in points allocated can be observed.

Table 2. Top 10 International experts

Top 10	**International experts**	**Mean**	**Std**	**Min**	**Max**
1	Transport of animals from different farms to the farm	14.4	10.7	5	30
2	Use of 3rd and 4th generation cephalosporin's	9.4	10.1	0	20
3	Adaptability/survivability of ESBL infected strains	8	11.5	0	25
4	Administration of antimicrobials in large groups of animals	7	9.75	0	20
5	Non-use of all in; all out management for building on the farm (continuous throughput)	5	7.07	0	15
5	Density of use, how often antimicrobials are used	5	5	0	10
7	Use of cephalosporin's in large groups of animals	4.8	6.72	0	14
8	Exposure to any surface water that may be contaminated with human or animal waste	4.4	2.61	0	7
9	Type of cephalosporin (XNL (ceftiofur) / CEQ (cefquinome)…)	4	8.94	0	20
10	The number of pigs treated with antimicrobials	3	6.71	0	15
10	Animal population resistance to specific ESBL spreading microorganisms	3	4.47	0	10
	Remaining	32			

The Netherlands

Just like the international experts, the selection of risk factors varies strongly between experts. Next to the top ten, 17 factors were selected over which 34 points of the 100 points were allocated. The number 2 and 3 in this top ten was not selected by international and Danish experts. In this top ten the points are more equally spread over the selection. The number 1, the use of 3rd and 4th generation cephalosporin's, was according to all experts a risk factor, that results in a lower standard deviation than the number 2 which was only selected by one experts but had received a lot of points. The Dutch experts all agreed on the fact that the use of 3rd an 4th generation cephalosporin's is an import risk factor as they all allocated points to this risk factor. On the other risk factors, the opinions are different.

Table 3. Top 10 The Netherlands

Top 10	**The Netherlands**	**Mean**	**Std**	**Min**	**Max**
1	Use of 3rd and 4th generation cephalosporin's	12	4.76	5	15
2	Poultry farms within 5 km (3 miles)	7.5	15.00	0	30
3	High animal density in pig units	6.8	5.38	0	12
4	Amino penicillin's used	6.3	7.50	0	15
5	Administration of antimicrobials in large groups of animals, > 50% of the stable/unit	5.3	6.08	0	11
5	Contact/collective stable with ESBL positive animals now or in the past	5.3	6.18	0	12
7	Transport of animals from different farms to the farm	5	10.00	0	20

7	The number of pigs treated with antimicrobials	5	10.00	0	20
7	Use of longacting injectable cephalosporin's	5	7.07	0	15
10	Use of cephalosporin's in large groups of animals	3.8	7.50	0	15
10	Administration of cephalosporin's through feed, injection or water	3.8	7.50	0	15
	Remaining	**34.2**			

6.1.3 Conclusions

The first selection of risk factors led to three top tens of risk factors. A relative large variation in the selection was found. This is probably due to the large variation of the background of experts. However, a few risk factors were selected by all the groups. The 'Use of 3rd and 4th generation cephalosporin's' was in all the top tens on the first or second place. Also the 'Use of cephalosporin's in large groups of animals' was in all the top tens. Closely related to this one, but less specific is 'Administration of antimicrobials in large groups of animals, >50% of the stable/unit', which was the number 5 in the Netherlands and number 4 in the international selection. Another important issue which was in two top tens was the use of amino penicillin's.

All the groups selected the following topics:

- Internal hygiene (transmission within the farm)
- Transport of animals from different farms to the farm
- Number of animals treated with cephalosporin's
- Administration of cephalosporin's through feed, injection or water

Transport of animals from different farms to the farm was the most important risk factor selected by international experts. In the Netherlands farms are allowed to receive animals from maximum 3 different farms since the outbreak of Classical Swine Fever. A difference in animal husbandry systems can be one of the reasons for the variation in selections by the different groups of experts. The differences in backgrounds and research where experts are involved in will also influence the variation of selections within all groups.

6.2 Expert meeting in the Netherlands – selecting and ranking risks

The expert meeting organized in the Netherlands was arranged to make a final selection of risk factors. Within the three groups (antimicrobial usage, internal bio-security and external bio-security) selection and ranking took place to be able to identify the main risk factors. Six experts were asked to allocate 100 points over every group of risk factors. The list of risk factors per group is given in the annex. After the selection and ranking, the experts were asked to type down the argumentation to select this specific risk factor.

6.2.1. Use of antibiotics

From the list of 26 risk factors, 17 factors were selected. The following two were clearly identified as the most crucial with 65 points of the 100. The standard deviation shows a strong variation in the points given to both risk factors.

Total amount antibiotics per time unit (per animal unit)

The total amount antibiotics used, determines the selection pressure and in that way the selective advantage for ESBL. Every use of antimicrobials (regardless of way of administration, type of antimicrobial or dosage) can form a risk for the spread of ESBL. This risk factor can be seen as a combination of several other risk factors selected in the list below including use of 3rd and 4th generation cephalosporin's, dose of used antimicrobial, density of use and others.

Exposure of the intestinal flora to antibiotics
The exposure of the intestinal flora to antibiotics is comparable to the first risk factor but more specific directed on ESBL because the exposure of the intestinal flora determines the selective advantage for ESBL in the gastrointestinal tract.

Table 4. Selection and points allocated to the group 'use of antibiotics'

Nr.	Group of risk factors: Use of antibiotics	Mean	Std	Min	Max
1	Total amount antibiotics per time unit (per animal unit)	45.0	46.4	0	100
2	Exposure of the intestinal flora	20.0	28	0	70
3	The more (unnecessary) treatments	3.3	5.2	0	10
4	Administration of antimicrobials through premix feed	3.3	5.2	0	10
5	Administration of antimicrobials in large groups of animals, > 50% of the stable/unit	3.3	5.2	0	10
6	Dose of used antimicrobial	3.3	8.2	0	20
7	Administration of cephalosporin's through feed, injection or water	3.3	8.2	0	20
8	Use of longacting injectable cephalosporin's	3.3	8.2	0	20
9	Density of use, how often antimicrobials are used	3.3	8.2	0	20
10	Oral administration of antimicrobials	1.7	4.1	0	10
11	Administration of antimicrobials through water	1.7	4.1	0	10
12	Use of cephalosporin's in large groups of animals	1.7	4.1	0	10
13	Use of 3rd and 4th generation cephalosporin's	1.7	4.1	0	10
14	Duration treatment with cephalosporin's	1.7	4.1	0	10
15	Duration of treatment in which the microorganism is exposed to the antimicrobial, long/prolonged time > 2 wks	1.7	4.1	0	10
16	Quantities of other antimicrobials used	0.8	2	0	5
17	Age of animal treated with cephalosporin's	0.8	2	0	5

<u>6.2.2. Internal bio-security</u>
Risks for the internal bio-security can be seen as the risks to prevent spreading of ESBL. From the list of 20 risk factors, 12 were selected and ranked. The following six factors are the most crucial risk factors for internal bio-security and received 84 of the 100 points. In this selection the points are more spread over the risk factors.

1. Movement of animals within the farm
Movement of animals within the farm can spread ESBL. In the public health movement of patients is seen as an important cause of the spread of ESBL.

2. Contact/collective stable with ESBL positive animals now or in the past
Contact with ESBL positive animals now or in the past and the application of collective stables in which ESBL positive animals can have contact can form a risk for the spread of ESBL. From the public health sector is known that patients can be carrier of ESBL positive bacteria in their intestinal flora for a long time and can form a reservoir for others. Contact with positive animals can lead to final introduction.

3. Spread by employees
Spread of ESBL producing bacteria by employees is possible when internal bio-security measures are not kept. It is considered to be a serious risk factor in maintaining internal bio-security.

4. Education level of employees
Education level of employees is related to all the risk factors because it determines the efficacy of the mentioned measures. Employees need to be well enough educated to be able to understand the importance of the measures. If measures are not complied with, risks for introduction and spread of ESBL are present.

5. Thoroughness of terminal hygiene procedures used to clean empty buildings
ESBL positive bacteria are able to survive for a long period of time in the environment, so thoroughness of terminal hygiene procedures used to clean empty buildings is needed. Insufficient hygiene procedures can be seen as a risk for the spread of ESBL producing bacteria into a new group of animals.

6. Use of collective instruments and materials
Use of collective instruments and materials can form a vehicle for ESBL positive bacteria and in that way spread ESBL positive bacteria in other animal groups.

Table 5. Selection and points allocated to the group 'internal bio-security'

Nr.	Group of risk factors: Internal bio-security	Mean	Std	Min	Max
1	Animal movement within the farm	18.3	9.3	0	25
2	Contact/collective stable with ESBL positive animals now or in the past	17.5	9.4	0	25
3	Spread by employees	17.5	19.4	0	40
4	Education level of employees	12.5	16.7	0	40
5	Thoroughness of terminal hygiene procedures used to clean empty buildings	10.8	9.2	0	20
6	Use of collective instruments and materials	7.5	6.1	0	15
7	Different animal groups (piglets, sows, finishing pigs) kept in one unit (one site production system)	3.3	5.2	0	10
8	Use of hospital pens, with return of animals	3.3	8.2	0	20
9	Extend of exposure to faeces	3.3	8.2	0	20
10	Possibilities of contact of other animal species kept on the farm with the pigs	2.5	4.2	0	10
11	High animal density in pig units	1.7	4.1	0	10
12	Inadequate ventilation	1.7	4.1	0	10

6.2.3. External bio-security
Risks for the external bio-security can be seen as the risks to prevent introduction of ESBL producing bacteria. The following six factors together received almost 62 of the 100 points that could be allocated to 27 risk factors for external bio-security. Differences in selections and ranking led to a total of 19 factors that received points.

1. Contact/collective stable with ESBL positive animals now or in the past
The most important risk factor in external bio-security is contact with ESBL positive animals now or in the past. From the public health sector it is known that patients can be carrier of ESBL positive bacteria in their intestinal flora for a long time and can form a reservoir for others. Contact with positive animals can lead to final introduction.

2. Pigs mixed from different 'sources'
Mixing animals from different 'sources' increases the risk for introducing ESBL positive animals/bacteria into the animal unit.

3. Transport of animals from different farms to the farm
Transport of animals from different farms to the farm brings together different backgrounds of farms which increases the risk for spread of positive ESBL bacteria. Transport of animals plays an important role in the spread of micro organisms including resistant variants.

4. Non-use of all in; all out management for building on the farm (continuous throughput)
A continuous throughput of animals in the farm, non-use of all in/all out management for animal units means that new animals will be contaminated once ESBL positive bacteria are present in the farm.

5. Introduction by people entering the farm
Introduction by people entering the farm can form a risk for introduction and spread of ESBL. Humans can be carrier of ESBL positive bacteria in their intestinal flora. Without taking sufficient hygiene measures, introduction of ESBL into the farm can take place.

6. Education level of employees
Education level of employees (arguments same as under 'internal bio-security')

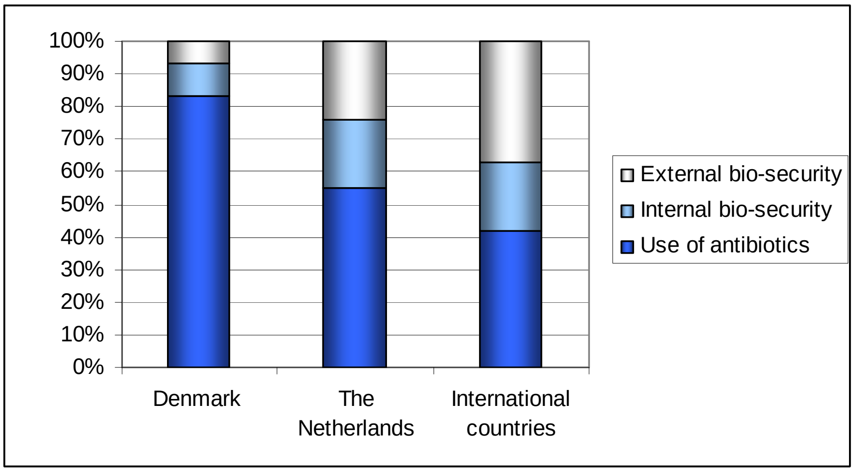
Table 6. Selection and points allocated to the group 'external bio-security'

Nr.	Group of risk factors: External bio-security	Mean	Std	Min	Max
1	Contact/collective stable with ESBL positive animals now or in the past	16.7	22.5	0	50
2	Pigs mixed from different 'sources'	14.2	16.9	0	40
3	Transport of animals from different farms to the farm	9.2	11.1	0	25
4	Non-use of all in; all out management for building on the farm (continuous throughput)	7.5	7.6	0	20
5	Introduction by people entering the farm	7.5	9.9	0	25
6	Education level of employees	6.7	16.3	0	40
7	Spread by the environment (excl animals	5.8	8.0	0	20
8	Exposure to any surface water that may be contaminated with human or animal waste	5.0	5.5	0	10
9	Feed as a possible source of ESBL	5.0	12.3	0	30
10	Exposure of pigs to wild life	4.2	4.9	0	10
11	Spreading of human sewage sludge as fertilizer on land around the farm	4.2	10.21	0	25
12	Poultry farms within 5 km (3 miles)	3.3	5.16	0	10
13	Transport of animals from one farm to different farms	1.7	4.08	0	10
14	Spreading of manure of animals from other (poultry/pig) farms on the land around the farm	1.7	4.08	0	10
15	Flooding events that may influence exposure to surface water	1.7	4.08	0	10
16	Employees/visitors entering farm without wearing company clothes	1.7	4.08	0	10
17	Use of same company clothing for the entire farm	1.7	4.08	0	10
18	Farmer of farm worker receiving antimicrobials	1.7	4.08	0	10
19	Exposure of feed stores to wild life	0.8	2.04	0	5

6.2.3. Conclusions
A strong focus in the internal and external bio-security is two issues; 1. Moving or mixing animals and possible contact with ESBL positive animals and 2. Spread by humans (employees or visitors). Environmental factors are selected but are more on the background when talking about the introduction and spread of ESBL producing bacteria. In the use of antibiotics, the risk 'total amount antibiotics per time unit (per animal unit)' is the most important risk factor, this simply because it contains most of the other risk factors. Some others are given points as well which nicely indicates some additional risks which were also important in the first selection.

6.2.5. Comparison of first and second selection
Grouping the risk factors of the first selection into 'use of antibiotics', 'internal bio-security' and 'external bio-security' and adding up the points allocated to this factors results in the following chart.

Figure 1. Chart of group distribution per country



Risk factors concerning the use of antibiotics received most of the points. This leads to the conclusion that the experts consider the use of antibiotics as the most important group of risk factors. Between internal and external bio-security differences are smaller, risk factors concerning external bio-security received in total a few more points.

Due to the grouping of risk factors in the final selection and the seven new formulated risks, it is more difficult to compare the results of both selection and ranking rounds.

Use of antibiotics
In the group of antibiotic use, the most important risk factor basically combines almost all the risk factors in this group. Other risk factors received only a few points compared to this. However, a few interesting other risk factors were selected which were also important in the first selection:
- Administration of antimicrobials in large groups of animals
- Administration of cephalosporin's through feed, injection or water
- Use of longacting injectable cephalosporin's

Internal bio-security
Internal bio-security includes three new formulated and combined risk factors. The risk factor 'internal hygiene' was selected in the first selection by all expert groups but was in the second selection taken out because it would include all the risk factors in this group (internal bio-security). 'Contact or collective stable with ESBL positive animals now or in the past' scored high in the first Dutch selection and now again. Interestingly enough 'education level' and 'thoroughness of terminal hygiene procedures used to clean empty buildings' were not the most selected issues when talking on the introduction and spread of ESBL in pigs in general, but when talking about the risks for the internal bio-security, they are in the top five.

External bio-security
For the same reason as in the group of internal bio-security, the risk factor 'external hygiene' was taken out. The final selection of crucial factors for external bio-security shows the most correspondence with the selected points on this topic in the first selection. 'Contact or collective stable with ESBL positive animals now or in the past' scored high in the first Dutch selection, the internal bio-security and in the final selection as well. Other risks related to external bio-security which were high in the first ranking include:

- The transport of animals from different farms to the farm (was in the top ten of both the Dutch as the international experts)
- Non-use of all in; all out management for buildings on the farm

6.3 Expert meeting in the Netherlands – brainstorm of ideas to manage risks

In the afternoon meeting the experts from the morning program were joined by farmers, veterinarians, a representative of the veterinarian pharmaceutical industry, Agropark specialists and other researchers. With a presentation on Agroparks and the input from the morning session as background, the fifteen experts were asked to come with ideas for managing the risk factors. A large diversity in ideas and solutions were generated in this meeting. Not all the solutions could be discussed during the meeting but it would be interesting to discuss further with the participants as valuable solutions were brought up. Below a summary of the total list of ideas is given, the complete list can be found in annex 5.

6.3.1 Use of antibiotics

Ideas to decrease the use of antibiotics included:

- Improve animal health
- Reduce selection pressure by
    - Limiting use of cephalosporin's
    - Limiting treatment in large groups of animals
    - Limiting administration through water or feed
- Convince farmers that investments in animal health can lead to lower use of antibiotics and better results
- Make protocols for treatment and prescription
- Make daily dosage per farm per animal category visible to give insight in the costs of the use and to reward low use

6.3.2 Internal bio-security

Ideas on internal bio-security were specified to the risk factors. The most important ideas are:

- Reduce animal movement to prevent internal spread
- Apply all in/all out management to reduce possible contact with ESBL positive animals
- Strict separation of animal units (kind of multi-site production system)
- Search for adequate cleaning systems
- Use a quarantine unit for treated and incoming animals
- Protocols for treatment to clarify procedures for employees
- Separate employees per animal unit to prevent internal spread
- Hygiene room, company clothes, hand disinfection and boots separate for every animal unit.
- Establish network to exchange successful measures and experiences
- Good communication about situation and methods to tackle risks
- Develop good method to thoroughly clean empty buildings
- Use different instruments and equipment in different animal units

6.3.3. External bio-security

- Close the farm, no introduction of new animals
- Keep animal groups together
- Make a route in the stable for entrance and exit, to easily shift the animals
- Prevent introduction by people by limiting entrance by visitors and strict hygiene protocols

6.3.4. General ideas

Next to the ideas given above, some ideas were not specific directed to a group. These risk factors include ideas for development of farms, research, cooperation and policy:

1. Farm

- Develop stables with bio-security as a point of departure
- Farm size should be large enough to have a closed farm with separate employees per animal unit

2. Research

- Develop quick diagnostics and improve monitoring system
- Monitor resistance
- Research after persistence of ESBL in farms to be able to develop adequate measures to clean farm buildings

3. Cooperation

- Establish networks with experts with different backgrounds (medical, food chain, food animal) to develop and evaluate measures, ideas and designs of stables
- Bring together research and practice to come to applicable solutions in practice

4. Policy

- A broad approach is needed, discuss this subject on EU level
- Finance research on ESBL and development of quick diagnostics in animal husbandry
- Be clear in communication to sector and community

7. Discussion

7.1 Applied method

Information on the risks of ESBL's when designing Agroparks and large-scale farms is needed to ensure food safety and safety of animal and public health. A list of risks and solutions on how to manage these risks was not available yet. Experimental research was not possible because agroparks do not yet exist and the costs for these kinds of researches are high. To get an impression on the risks and possible solutions, the method, self explication approach in conjoint analysis, was chosen. This, in comparison to expert interviews, improves the reliability, repeatability and validness of the research by quantifying expert opinion. Conjoint analysis has been used in several studies and showed that it can be a useful tool in the veterinary field.

Because it is difficult to assess whether the impact of the risk factors scored by the experts is similar to the true impact of the factors in practice, experts with different backgrounds (incl. medical, food chain and animal husbandry experts) were invited to share their knowledge and limit the possible gap between expectations and practice. Fifteen experts from five different countries were involved in making the list of risk factors and selecting the most crucial risk factors. The variety in opinions led to an extended list of risk factors of 112 factors and different selections.

The advantage of this method is that large amounts of factors can be handled relatively simple. However, selecting risk factors and dividing points does require a lot of consideration from the experts.

The method does have its disadvantages. One of the disadvantages of the method is that inter-correlation between attributes was difficult to take into account with this system (Green et al. 1990). Combining different risk factors solved this problem partly. Another disadvantage is that if someone gives a lot of points to one factor, this has a strong influence in the results, a spread of points over different important factors may for that reason not be noticed. The influence of every expert is relatively large due to the limited number of experts per group.

The used method was for two invited experts a reason not to cooperate in this research. The reason for not cooperating was that in their opinion risks should be investigated by research on underlying processes, not with scores of experts. This can not be denied, however, considering the situation in which we are developing large scale farms and Agroparks, its better to hear what experts see as risks in this development, and how to manage it than to deny the possible risks in designing Agroparks and large scale farms, while waiting for the results of this research.

7.2 Results in relation to findings of previous research

When looking at the entire list of risk factors several main topics can be derived from it;

1. use of antibiotics
2. farm management
3. people
4. environment

Although the final selection made a different grouping of factors, this is the clearest division to relate the most crucial risk factors to findings of previous research. In figure 2 possible relationships between the different risk factors are given.

7.2.1. Use of antibiotics
Selective pressure refers to the environmental conditions that allow organisms with novel mutations or newly required characteristics to survive and proliferate. (Tenover et al. 1996) Strong selection pressure exerted by antimicrobial use, especially with newer generation beta lactam antibiotics, promotes ESBL emergence and subsequent spread. However, transfer of resistance plasmids can also occur in the absence of antibiotics. (Kruse et al., 1994) It also stimulates further evolution of ESBLs by accumulation of other mutations with a large variety of effects on beta lactamase structure and activity. (Gniadkowski, 2008) The intestines are a place where, because of the enormous amount of bacteria, the effects of selection pressure through the use of antibiotics are observed. A continuous flow of new bacteria through food is added to the intestinal flora and colonize and exchange genetic material. When using antibiotics all sensitive bacteria will be killed or slowed down and the resistant bacteria will grow because of the selective advantage.

The total amount antibiotics used, determines the selection pressure and in that way the selective advantage for ESBL. For that reason it was selected as the most important risk in the group of use of antibiotics. Every use of antimicrobials (regardless of way of administration, type of antimicrobial or dosage) can form a risk for the spread of ESBL, however specifically the exposure of the intestinal flora influences the selective advantage for ESBL. When, for example, cephalosporin's are used, selection of ESBL – producing *E. coli* takes place in the intestinal flora of animals. (Cavaco et al., 2008; Jorgensen et al. 2007) This is one of the reasons why the use of cephalosporin's was indicated as an important risk factor. The critical importance of cephalosporin's for human medicine also contributes to importance of this risk. (WHO, 2007) The type of cephalosporin was also indicated as a risk. Differences in selective effects of three types of cephalosporin's have been shown. The effects also persisted beyond the withdrawal time recommended for these cephalosporin's which might lead to meat contamination in slaughterhouses. (Cavaco et al. 2008)

The adaptability and survivability of ESBL infected strains was by international experts indicated as an important risk factor. Differences in the adaptability and survivability of the ESBL infected strains occur, it can even disappear after a period of time. (Burch, personal comment) The epidemiology of ESBL-producing bacteria is becoming more complex. (Pitout and Laupland, 2008) Strong selection pressure stimulates evolution of ESBLs by accumulation of other mutations with effects on the structure and activity of beta lactamase. Structural modifications of resistance genes by mutation and recombination, together with a multitude of events that stimulate their mobility and expression, allow microorganisms to survive in environments saturated with antimicrobial agents of various types and generations. (Gniadkowski, 2008) This process can form an additional risk for the spread of ESBL, efficacy of treatments and diagnosis of ESBLs.

7.2.2. Farm management
Animal movement and contact with ESBL positive animals was seen as crucial risk factors. As many ESBL genes are located on plasmids, transmission of bacteria can take place. (Bradford, 2001). For that reason, the movement of animals can easily lead to the spread of ESBL-producing bacteria. Contact with ESBL positive animals now or in the past and the application of collective stables in which ESBL positive animals can have contact, can form a risk for the spread of ESBL. As Liebana et al. indicated; horizontal plasmid transfer between strains as well as horizontal gene transfer between plasmids can contribute to the spread of resistance. Genes can be

exchanged between different microbial populations of different origins. (Liebana et al., 2006)

Transport of animals from different farms to the farm and mixing animals was indicated as a risk. (Burch et al, 2008) Also a continuous throughput of animals can lead to the spread of disease when susceptible pigs enter a contaminated animal unit. The application of all in/all out housing systems together with good hygiene is in increasing extend applied in farms and is seen as successful to reduce the spread of disease from one group of pigs to another. (Burch et al., 2008) It breaks the disease cycle by preventing the exposure of (new, incoming) pigs susceptible to infections to pigs (already present) carrying clinical disease. (Carr, 2006) Adequate stable hygiene measures and ventilation can make a good step forwards in providing the optimal environment for the pigs to live, without drafts, moisture, gases and pathogens. (Burch et al, 2008)

Specialization in labor, buildings and feed has led to specialized production; farms with only sows, piglets or fatteners. Transport is needed to move the animals from one farm to the other and often goes together with mixing animals. For that reason specialized farms can contribute to the spread of diseases. A closed chain (from sow to fattening pig) can contribute to a better maintenance of bio-security, however strict separation of animal units is necessary to reduce the risk for possible internal spread. Specialized labor can be employed for the different production units within a company to prevent transmission of bacteria from one production stage to another.

7.2.3. People

Introduction of ESBL into a farm can be caused by persons as the prevalence of ESBL in the community is increasing. (Livermore et al., 2007; Cantón and Coque, 2006; Pitout and Laupland, 2008) People entering the farm are important risk factors as they can carry infectious agents on their skin and clothes. Next to not wearing company clothes, use of the same company clothing when entering different production stages can transfer ESBL positive bacteria and is for that reason identified as a risk factor for the introduction spread of ESBL. Going through a shower, clothes changing system and a down time without pig contact are frequently applied measures. (Burch et al., 2008) Also visiting other farms, having family working in the public health sector and receiving antimicrobials as a farmer or farm worker were mentioned as risk factors. Persons can introduce or spread ESBL by not taking in account these necessary bio-security measures. The importance of the measures needs to be understood by the farmer and his employees. That is why the education level of the employees plays a key factor in maintaining both internal and external bio-security.

7.2.4. Environment

In an American study on the antimicrobial resistance in swine waste, samples were collected from the swine feed, each stage of waste collection and treatment, nearby ground and/or surface water, and manure-amended soils. This study found several tetracycline resistance determinants in feed at all of the farms, including an organic farm. This is why animal feed should not be left out of consideration as a possible source of antimicrobial resistance genes. (Jindal et al, 2006) Wildlife might play a role in this.

Several studies have detected ESBL-producing *E. coli* isolates in wild animals (including small mammals, birds, deer and foxes). (Costa et al, 2006; Kozak et al, 2009) Wild small mammals living close to food animal farms have higher rates of resistance and are more frequently multiresistant than *E. coli* isolates from natural areas, possible due to exposure to resistance from livestock or animal feed with antimicrobials. (Kozak et al., 2009) Wild life can in this way transfer resistance from one farm to the other.

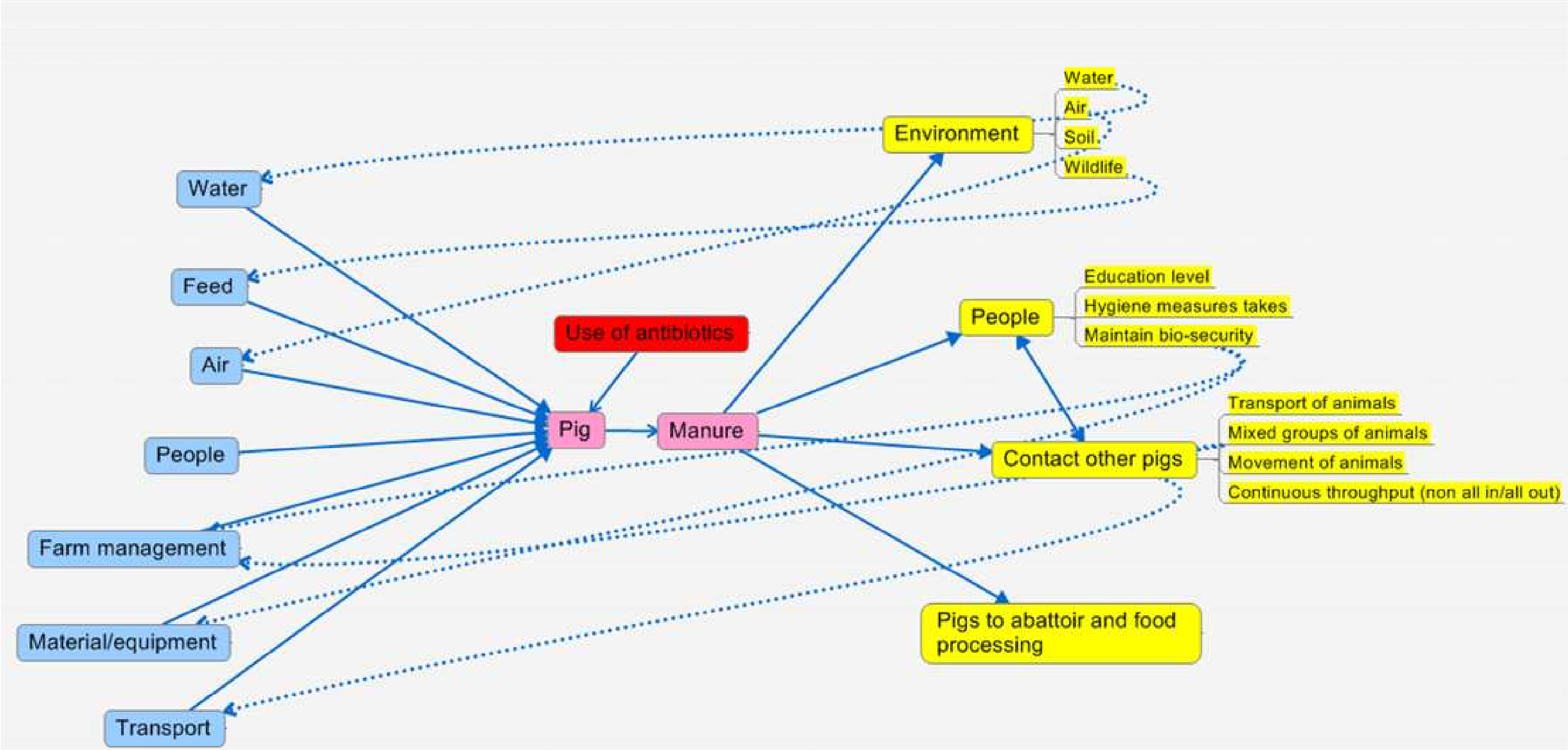
Wild life can also be exposed to antimicrobial resistance through the environment. The presence of high levels of antimicrobial resistance not only in swine manure but throughout the entire waste treatment process makes the spread of manure of animals on farm land a risk because it may contribute to an environmental reservoir of resistance. (Jindal et al., 2006) Antimicrobial residues have also been found in water samples; the application of animal waste to agricultural fields as fertilizer is seen as a source of the antimicrobial residues in water resources. (Campagnolo et al., 2002)

In water, bacteria with different origins (human, animal and environmental) come together and exchange of genes can take place. (Baquero et al., 2008) Exposure to any surface water that may be contaminated with human or animal waste is considered to be an important risk factor for the introduction and spread of ESBL producing bacteria.

Environmental transmission of antimicrobial resistance can also take place by air. Bacterial concentrations with multiple antibiotic resistances were found inside and at least 150 m downwind outside farms. (Green et al., 2006) Inhalation of air from pig farms may serve as an exposure pathway for the transfer of multidrug-resistant bacterial pathogens from swine to humans. (Chapin et al., 2005)

Also the close proximity of poultry farms can form a risk because the occurrence of ESBL-producing organisms in poultry is in the Netherlands a recent concern. Poultry as a reservoir of ESBL genes may form a human health hazard as well as risk for other animals. (Smet et al., 2008; Girlich et al., 2007).

Figure 2. Relations risk factors



Overview of (possible) relations between different risk factors (inputs and outputs) of pig production. Not all risk factors have been put in. At the left the inputs (blue) are given and can bring in ESBL positive bacteria and contaminate pigs (pink). At the right, the outputs are given in yellow. To explain an example: People (labor) are an input for pig production; they can introduce ESBL in the stable. Through pigs and manure people can come in contact with ESBL in the stable. The people also have contact with other animals and are therefore related outputs. Depending on the education level and hygiene measures taken, bio-security is maintained which influences the hygiene of buildings and materials equipments as inputs.

7.2.5. Agroparks in relation to the risk factors for the use of antibiotics
For all the risks that were selected, argumentation has been found. The selection pressure plays an important role in this group of risk factor. Decreasing the selection pressure is the most important solution to reduce the selective advantage for ESBL and possible evolution. Treatment of large groups of animals as well as administration through feed and water are seen as additional risks. Although it is not a factor directly important for introduction and spread of ESBL in a pig farm or Agroparks, the adaptability and survivability of ESBL is important as it can relate to the possibilities to manage the problem of ESBL.

The most important task for Agroparks on this topic is to improve animal health to reduce the use of antibiotics and the selective advantage for ESBL. Individual treatment through injection is preferred but requires time and labor.

7.2.6. Agroparks in relation to risk factors for the internal bio-security
Agroparks and large scale farms are likely to apply the principle of all in – all out. This prevents exposure of incoming animals to present pathogens. The large size of an Agropark enables separating animal units with different employees per unit. A reduction of the possible spread of ESBL-producing bacteria between units can be expected. Separate equipment and materials should be used per unit or thorough disinfection methods can be applied when materials are used in different animal units. There is an intense need of continuous monitoring in the total chain in the Agropark to safeguard animal and public health.

7.2.7. Agroparks in relation to risk factors for the external bio-security
Agroparks and large scale farms are assumed to be closed farms without introduction of new animals. For that reason the risk by transport of animals from different farms does not apply for Agroparks. All in – all out management will also reduce the risk for introduction of ESBL positive bacteria in an animal unit and contaminating the animals that are still present in the unit. Because Agroparks are a new way of production, visitors can be limited by building a visitor centre. Entrance of veterinarians will still be needed. Specific Pathogen Free (SPF) farms already apply hygiene measures that can reduce spread and introduction of ESBL positive bacteria. These measures are known and can be implemented in large scale farms and Agroparks as well to reduce the risk for introduction of ESBL.

7.3 Recommendations for further research

The discussion on the finding related to previous research is very broad but comes back to one point, the use of antibiotics. Future research should have a focus on ways to improve the health of pigs and decrease the use of antibiotics, with applicable and manageable solutions for the practice in pig farms. Designing a farm out of the principle of bio-security might lead to new interesting ways to keep animals healthy with a reduced use of antibiotics.

As we are now in a situation where the resistance levels increase as well as the use of antibiotics, close monitoring of resistance levels is critically important. The strong selection pressure might stimulate further evolution of ESBL and affect the structure and activity of beta lactamase. Good diagnostic methods need to be developed to monitor the spread of resistance genes. Also development of quick diagnostics for the animal husbandry sector could be helpful to have a controlling system when the pigs are entering the farm.

Further detailed study is needed to gain insight in the complex relation of the occurrence of ESBL's in both animal and human bacteria and their possible connections. Also the environment as a reservoir of ESBL positive bacteria should be investigated. Identification of the selection criteria for ESBL is needed to make a further analysis of the risks for introduction and spread of ESBL producing bacteria.

7.4 Contribution of this research to literature

Field surveys and experimental research will be needed to support the list of risk factors and their solutions because this research might not have revealed the true impact of the risk factors and the true solutions for the risk factors. However, this method with expert opinion investigation led to a gathering of knowledge, a list of risk factors with possible solutions and highlighted the emerge of ESBL. The results can be used when developing Agroparks and large scale farms to make a step forwards in ensuring food safety and safety of animal and public health. This makes it a useful and practical applicable contribution to the knowledge on this topic.

8. Conclusions on risks and its management of introduction and spread of ESBL in large scale animal husbandry

To ensure food safety and safety of animal and public health when designing Agroparks and large scale farms, managing the risks for introduction and spread of ESBLs is necessary. To be able to think of management solutions for the risks, a list of crucial risk factors and its protocols to prevent them is needed. Furthermore it is interesting to take a look at how the Agropark Nieuw Gemengd Bedrijf (New Mixed Farm) is going to manage the topic 'antimicrobial resistance'.

8.1 The risk factors for introduction and spread of ESBL in pigs

The opinions on the risk factors for introduction and spread of ESBL vary a lot between the different experts probably because of different backgrounds and research where experts are involved in. As presented in the discussion, there are many aspects influencing the occurrence of ESBL. This means that there are no fixed answers to the research questions: 'What are the risk factors?' and 'What are the ideas for managing these risks?'. The risk factors as given below do given an impression on what issues are important to reduce the possible risks for introduction and spread of ESBL.

Based on the first selection, the majority of the points were allocated to risk factors concerning the use of antibiotics. As the use of antibiotics determines the selection pressure and in that way forms a risk for the evolution and spread of ESBL, the internal and external bio-security can 'only' help to take measures to prevent introduction and spread.

<u>8.1.1.Use of antibiotics</u>

In the use of antibiotics the most crucial risk factors are:

1. *Total amount antibiotics per time unit, per animal unit*
2. *Exposure of the intestinal flora to antibiotics*

<u>8.1.2. Internal bio-security</u>

The internal bio-security includes the ways of transmission of ESBL producing bacteria within the farm, the risks for spread of ESBL.

1. *Animal movement within the farm*
2. *Contact/collective stable with ESBL positive animals now or in the past*
3. *Spread by employees*
4. *Education level of employees*
5. *Thoroughness of terminal hygiene procedures used to clean empty buildings*
6. *Use of collective instruments and materials*

<u>8.1.3. External bio-security</u>

External bio-security focuses on the risks for transmission from outside to the farm, the introduction of ESBL producing bacteria.

1. *Contact/collective stable with ESBL positive animals now or in the past*
2. *Pigs mixed from different 'sources'*
3. *Transport of animals from different farms to the farm*
4. *Non-use of all in; all out management for building on the farm (continuous throughput)*
5. *Introduction by people entering the farm*
6. *Education level of employees*

8.2 Possible solutions on risk management for ESBL introduction and spread of pigs in Agroparks and large scale pig farms

Improvement of animal health, disease prevention, is a key to reach a reduction of antimicrobial use. Applying High Health and Specific Pathogen Free principles can help with improving disease prevention. Also good quality feed and a good climate can contribute to this. By reducing the use of antimicrobials, the selection pressure and the risk for evolution and spread of ESBL will decrease. The characteristics, dose and concentration and way of administering the antimicrobial all have influence on the selection pressure. In case antimicrobials need to be used, individual treatment is preferred. Administration of antimicrobials through feed and water should be prevented to reduce the risk of exposure of employees and wild animals, like birds and rodents, to antimicrobials. Treatment with cephalosporin's should be limited as far as possible. Early diagnostics and a quick and adequate treatment with accurate diagnosis help to have an accurate treatment.

Designing animal units with the principle of bio-security as a starting point can help finding new solutions for hygiene, cleaning and optimal animal health. Adequate stable hygiene measures and ventilation is needed to provide the optimal environment for pigs to live. "All in – all out" principles are advised as well as strict separation of animal units (sows, piglets and fatteners). Reducing possible contact with ESBL positive animals is crucial and can be reached by reducing necessary animal movements and transport. Multi site production with physically separation of animal units has the advantage of reduced chance on transmission of bacteria by e.g. air or employees but has the disadvantage of necessary transport of animals between units. A closed system will minimize the introduction of new animals. When new animals are introduced, a thorough "front door approach" with quick diagnostics and quarantine units can be a good way to prevent contact with possible ESBL positive animals. Different equipment and materials should be used per animal unit or a throughout disinfection method should be applied when materials are used in different animal units.

Different employees per animal unit are a good way to prevent spreading of ESBL positive bacteria from one unit to the other by humans. When entering an animal unit hygiene rooms need to be present to shower, change clothes and disinfect hands. Protocols for treatment and hygiene can help employees to have a guideline in their work. Educating employees as well as students to understand the different measures is important for awareness of the impact and maintaining bio-security. Entrance of visitors should be reduced to the minimum. A hygiene protocol can determine whether a visitor is allowed or not. An one to three days down time without pig contact for visitors is mentioned and applied in SPF farming as an additional disease avoidance policy next to showering and changing clothes. (Burch et al., 2008; Carr, 2006) Contact with other animal species, like poultry, should also be taken into consideration.

The large scale of Agroparks enables investments in high tech solutions to reduce the risk of transfer of multidrug resistant bacterial pathogens through the air and other possible pathways. Also incoming air needs to be filtered as the proximity of other farms can form a risk for the introduction of ESBL in the animal units. To manage environmental risk factors, an adequate cleaning system for water should be applied to minimize the risks of introduction by drinking water. Also for the outputs, waste water and manure, the farm should have a cleaning system to prevent spread of antimicrobial resistance to the environment. Proper "vermin" control, prevent contact with other animal species and prevent contact of animals to the feed storage should reduce the risk of introduction and spread by other animal species. As air has also been indicated as a pathway for transfer of multi-drug resistant bacterial pathogens close proximity of poultry, pig and other animal husbandry farms should be prevented. If different animal species will be combined in an Agropark, strict separation of animal units, employees, equipment and transport is necessary to reduce the possible spread of ESBL producing bacteria.

8.3 Nieuw Gemengd Bedrijf

How is the pig unit of the future Agropark, Nieuw Gemengd Bedrijf (NGB), planning to take care of these risks?

8.3.1.Use of antibiotics

By applying Specific Pathogen Free (SPF) principles in the management of NGB, they hope to improve the animal health. Also vaccination will be used to further improve the immunity of the animals. To decrease the selection pressure a minimum use of antibiotics is their goal. Administration of antibiotics through water or feed often takes place in large groups of animals which leads to selection pressure in a large group of animals. Administration of antibiotics in NGB, will take place as much as possible through injection instead of water or feed to reduce the selection pressure and possible spread of ESBL.

8.3.2. Internal bio-security

Strict separation of piglets, sows and fatteners with different buildings and employees for each of the three animal groups will be applied. Every unit has its own employees and hygiene room including showers and company clothes. Employees are allowed to work on the farm as long as they do not visit or work on other pig farms. They have to shower when they arrive and when they leave. These measures are taken to reduce the possible spread by employees. Employees will be educated within the farm, qualified people are needed with motivation and knowledge. Continuous control on execution of established protocols is essential to keep up standards. Management with high bio-security management is necessary.

Only when the piglets are moved from the sow to the piglets building they will go outside, other movements will be done inside the building. Also the loading of fattening pigs to the truck will take place inside. Sick animals will not be moved to hospital pens but will be euthanized to prevent spread of infection through the hospital pens and moving the animals to the hospital pen. Materials or equipment entering or leaving a unit will be disinfected with among other things UV boxes.

8.3.3. External bio-security

NGB will be a closed farm, without introduction of new animals except for the occasional introduction of a new boar. The goal is to keep the groups of animals as much as possible together from their birth till the end. All in – all out management will by applied for the animal units. The veterinarian will be asked to visit the farm on Monday morning, to make sure he/she did not visit a pig farm in the last 72 hours. (Carr, 2006) Visitors will be limited to the minimum.

Waste water and manure will be processed and exported. In the area are several large poultry and pig farms which can be found within a zone of 5 km. The nearest pig farm is located at 1 km from the farm. The new poultry farm of NGB will be located 1 km from the pig farm. Exposure of pigs and feed stores to wild birds or rodents or other wild life can form a risk. The retention pool attracts birds (ducks etc.) which can transfer ESBL positive bacteria and diseases. Also wild boars nearing the province of Limburg can form a risk in the transmission of diseases and ESBL.

8.3.4. Conclusion

The goals of the pig farm of NGB: minimum use of antibiotics, minimum entrance of visitors, education of employees inside the farm, closed farming with multi site principles and all in – all out management, are big steps in managing the crucial risk factors for the introduction and spread of ESBL. The size of the farm offers a lot of opportunities to manage several issues and make it financially feasible too in the long run. When these ideas are implemented, NGB is expected to be able to manage several of the mentioned risk factors and can therefore have a lower risk for the introduction and spread of ESBL. Once NGB is running it will be essential to monitor the antimicrobial use, the resistance levels and the production and financial results.

9. Recommendations

9.1 Agroparks, large scale farms and Nieuw Gemengd Bedrijf

Agroparks with large scale farming and a new farming system with closed production chains and certified quality management, can offer new opportunities to manage risk factors for the introduction and spread of ESBL spreading bacteria and other resistant pathogens. Precondition is superb quality management in the total value chain and well educated personnel. Inside multi-site production in which animal units are strictly separated is needed to decrease possible transmission of ESBL. Increasing the space between units (both pig and poultry) can help to decrease possible transmission by employees or by air. Maintaining distance between pig and poultry farms is important to prevent spread because a rapid increase in the occurrence of ESBL-producing organisms is in the Netherlands ESBL predominantly observed in poultry and poultry meat products. (MARAN 2007). Also distance between other animal husbandry is necessary as for example in the UK the most common occurrence of ESBL's has been in the cattle side, primarily in calf production. (Burch, personal comment)

A closed production chain offers a lot of possibilities for managing risks for spread and introduction of ESBL, not only in the farm but also during slaughtering and food processing. Because Agroparks cluster different agro-production chains with spatial combination of agro-processing, agro logistics and trade, less transport is needed and agro-processing will only process products produced within the Agropark. This decreases the risk of contamination (with bacteria from products coming from other farms) of food products during processing. On the other hand, when contamination occurs, the consequences need to be dealt with in the total chain and will be very large. For that reason continuous monitoring in the total chain in the Agropark is necessary to safeguard animal and public health.

In Agroparks, re-use of waste and by-products is one of the goals. Adequate treatment, e.g. heating, is needed to solve possible transmission of ESBL producing bacteria through the Agropark when waste products are re-used. When adequate measures are taken within the Agropark, managing the risks for introduction and spread of ESBL producing bacteria is expected to be better possible in Agroparks than in traditional production. However, a lot of different issues concerning external and internal bio-security can be managed, but it is good to realize that several characteristics on the molecular level are not manageable.

Designing animal units out of the principle of bio-security can help finding new solutions for hygiene, cleaning and optimal animal health. Improvement of health will lead to improvement of production and financial results. Improvement of health will go hand in hand with a reduced use of antibiotics. A reduction in the use of antibiotics is necessary to limit the selection pressure and the risk for evolution and spread of ESBL. Administrating antibiotics to large groups of animals through water or feed should be prevented. Educating employees and students to understand the different measures is important for awareness of the impact and maintaining bio-security.

Next to managing the risks for introduction and spread within the farm, also the responsibility has to be taken to prevent spread to and from the environment and the community. As Agroparks are more than only pig production, importance of bio-security measures and proper hygiene also apply during the whole value chain, as the abattoir slaughtering and food processing. Also manure and waste water need careful treatment.

Agroparks is a concept that requires a multi-disciplinary approach, but also within the animal husbandry discipline involving different parties when designing an Agropark can help to overcome many obstacles managing ESBL within a farm will face. The combination of (implicit) practical knowledge with (explicit) scientific knowledge can form a golden formula.

9.2 Other recommendations

Agroparks are not the only place where ESBL needs to be managed. Important is to realize that the community, animals as well as the environment are reservoirs of ESBL. Clear professional communication for different types of target groups about what is known about possible connections between those reservoirs is needed to prevent unnecessary commotion. Raising awareness of ESBL is needed to convince different parties that investments to manage this problem are necessary to prevent future problems with therapy failure.

Networks and information evenings to exchange ideas and solutions for managing animal health and more specific topics like ESBL should be set up to communicate and learn about this topic. The topic should also be discussed in agricultural education to work on the awareness of this issue of future farmers. By gathering researchers, veterinarians and farmers, applicable and manageable solutions for pig farming can be developed. In this research the willingness of the different parties to cooperate on this topic was there and should be applied also in the future.

Antimicrobial resistance and specifically ESBL, is not limited to the borders of the Netherlands and therefore needs a broad approach. International cooperation on this topic takes often place and can bring together different researches, perspectives and ideas. Further cooperation of the animal and public health sector can results in more insight in the situation, management solutions and future of the problem. Investments from the government as well as from the different sectors are needed to do further research and implement ideas. In the discussion ideas for further research were mentioned.

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11. Annex

Annex 1. List of involved persons

Experts involved in the ESBL expert meeting in the Netherlands and/or involved in the email sessions

1. Prof. Dr. D.J. (Dik) Mevius, CVI
2. Drs. C.M. (Cindy) Dierikx, CVI
3. Dr. M.A. (Maurine) Leverstein – Van Hall, UMCU, RIVM
4. Dr. J. (Jobke) van Hout, Gezondheidsdienst voor Dieren
5. Drs. W.M.T. (Resie) Oude Luttikhuis, Van Hall Larenstein
6. Drs. P.J.A.M. (Peter) Smeets, Alterra
7. Dr. M. (Manon) Swanenburg, CVI
8. Drs. E.M. (Els) Broens, WU, Leerstoelgroep Kwantitatieve veterinaire epidemiologie

Experts involved in the expert meeting 'management of ESBL'

1. Drs. M.J.M. (Madeleine) van Mansfeld, Alterra
2. Ing. B. (Ben) Wit, VWA
3. Dhr. G. (Gertjan) Vullings, Varkenshouder, Nieuw Gemengd Bedrijf
4. Drs. G. (Gerard) van Eijden, KNMvD, Vakgroep Gezondheidszorg Varken
5. Drs. B.G.M. (Björn) Eussen, FIDIN
6. Ir. J.B. (Bennie) van der Fels, Animal Science Group (WUR)
7. Dr. Ir. J. (Jan) Broeze, Agrotechnology & Food Sciences Group (WUR)
8. Dhr. J. (Jaco) Geurts, Nederlandse Vakbond Varkenshouders (NVV)

Experts involved in the ESBL expert meeting in Denmark
National Food Institute, Technical University of Denmark

1. Prof. F.M. (Frank) Aarestrup
2. Dr. H. (Henrik) Hasman
3. Dr. L.M. (Lina) Cavaco

ESBL experts from outside the Netherlands and Denmark involved in the email sessions

1. Dr. L.C. (Lucy) Snow, Centre for Epidemiology and Risk Analysis, Veterinary Laboratories Agency, UK.
2. Dr. C. (Chris) Teale, Veterinary Laboratories Agency, UK.
3. Dr. S.A. (Scott) McEwen, Population Medicine, University of Guelph, Canada.
4. Dr. C. (Christina) Greko, Department of Antibiotics, National Veterinary Institute, Sweden.
5. Dr. D.G.S. (David) Burch, Octagon Services Ltd, UK.

Others involved during this research

1. Ir. M.M.B. (Marion) Bogers, Alterra
2. Dr. B. (Bas) Engel, Leerstoelgroep Wiskundige en statistische methoden, WUR
3. Dr. Ir. H.J. (Ine) van der Fels, RIKILT – Instituut voor Voedselveiligheid
4. Dr. Ir. P. (Piet) Sterrenburg, RIKILT – Instituut voor Voedselveiligheid

Annex 2. Future Agropark Nieuw Gemengd Bedrijf

Four innovative entrepreneurs are working together with knowledge institutes on Nieuw Gemengd Bedrijf (New Mixed Farm): a regional cooperation of plant and animal production companies that will connect their waste and material flows. It will be a spatial clustering of existing holdings in this region with intensive exchange of remainders and byproducts. By reduction of the transport kilometers, environmental profit is created. Improved animal welfare will be achieved by optimizing the stables and minimize transport. Waste products will be processed and can bring in money. The cooperation includes among others the Wageningen UR, province of Limburg, Ministry of Agriculture, Nature and Food Quality – South and the municipality Horst aan de Maas.

Illustrator: Erik Visser; Architect: TRZIN

Farm design

The future Agropark, Nieuw Gemengd Bedrijf, a combination of poultry and pig farms is made. The pig farm will have 2500 sows, 10.000 piglets and 20.000 fatteners. The farm will most likely be a Specific Pathogen Free farm, which includes several specific hygiene and animal health measures.

The American idea of Multisite production will be applied, meaning that the three groups of animals will be separated. Not on different locations like in the USA but on one location. Strict separation of the 3 groups of animals will be made with different building and employees. Every unit will have their own employees and hygiene room including showers and company clothes.

The goal is to keep the groups of animals as much as possible together, no merging of animals if possible. The group size for the piglets will be 17 piglets per group, the weaning age will be between 21 and 28 days. For the fatteners the group size will be 15 animals per group and about 40 sows per group in the sow unit.
For the sows and fatteners two new units will be build with each two floors. At the moment five (one floor) stables are used for fatteners, these will be reconstructed for keeping piglets.

The farm will use semen of own boars and use own breeding sows. The farm will be closed for new animals except for the introduction of a new boar.

Combined biological and chemical air scrubbers will be used in the animal units Materials or equipment entering or leaving a unit will be disinfected with among other things UV boxes.

The feed will be mixed in the farm and will be pumped through pipes to the units. Water will be pumped up from a deep source, clean water from 180 meters deep.

Transport of animals
When the piglets are 8 kg, they will be transported to the piglets unit, when they are 23 kg they will be moved to the fattener unit. The movement from the piglet to the fattener unit can be made by letting the animals walk to the other stable. This movement takes place within the stable, they do not have to go outside. Moving the piglets from the sow to the piglet unit will be done by a kind of train with wagons to keep the groups together. The fatteners will be loaded inside the stable into the truck, they do not need to go outside.

Sick animals will not be moved to hospital pens but will be euthanized to prevent spread of infection through the hospital pens and moving the animals to the hospital pen.

Antibiotics
The farm will use the principles of High Health which means for them, minimum use of antibiotics. When it is used, it will be used curative. Their goal is to prevent with vaccinations rather than cure or treat with antibiotics. Injection on animal level and perhaps sometimes through feed or water. Blood research is already used frequently but sometimes you need to treat before you have the results. Of course the treatment will be evaluated afterwards. A treatment protocol is available from the veterinarian. No antimicrobial agents are excluded for used, but they are in favor of not using agents which are used in the human public health sector.

Employees
Family Vullings will live on the farm but this part is separated from the farm itself. Other employees will live at other places. Employees are allowed to work on the farm as long as they do not visit or work on other pig farms. They have to shower when they arrive and when they leave. It is possible that further requirements will be made. Every animal unit will have its own color overall what makes it possible to immediately observe when an employee visited another production unit.

Employees will be educated within the farm, qualified people are needed with motivation and knowledge. They want to deliver a good employee environment because they want continuance in the group of employees. Because often employees have family working in the public health care it is not possible to ask for employees without family in the public health care. Good hygiene measures need to be taken.

Farm access
The veterinarian will be asked to visit the farm on Monday morning, to make sure he/she did not visit a pig farm in the last 72 hours. The veterinarian will work from sows, to piglets towards the fatteners.

Visitors will be limited to the minimum. Perhaps a video info centre will be made. No visitors in the stable.

Companion animals are not allowed inside the animal units.

Poultry
In the poultry farm 1.200.000 broilers will be kept and 70.000 broiler breeders on 1 km distance from the pig farm. The complete chain will be present. There will be an abattoir, only for the poultry of this farm. The cooperation with the poultry farm is especially directed on the processing of the manure.

Manure
The manure of the pig farm will be transported by pipeline and will be digested and composted together with the manure of the poultry and will be exported. Waste water will go into the manure through the manure pipes and will be processed as well.

Area
In the area are several large poultry and pig farms can be found within a zone of 5 km. The nearest pig farm is located at 1 km from the farm.

Wild pigs are heading towards Limburg from Germany, this is worrisome. Exposure of pigs to wild birds or rodents or other wild life can form a risk. The retention pools attracts birds (ducks etc.) which can transfer bird flu and close down the entire company.

Annex 3.

Table 7. Complete list of risk factors for first selection

	Risk factors for introduction and spread of ESBL-producing bacteria in pig farms
1	External hygiene (transmission to the farm)
2	Internal hygiene (transmission within the farm)
3	Transport of animals from different farms to the farm
4	Transport of animals from one farm to different farms
5	Import of equipment into the unit used in only one animal unit without disinfection
6	Import of equipment into the unit used in different animal units/farms without disinfection
7	Type of farm: Organic
8	Type of farm: Conventional
9	Type of farm: High Health/SPF
10	Farm size
11	Direct access for feed deliveries
12	Direct access for deliveries of goods/equipment
13	Presence of main road near to farm
14	Pig farms within 5 km (3 miles)
15	Poultry farms within 5 km (3 miles)
16	Exposure of pigs to wild birds or rodents or other wild life
17	Exposure of feed stores to birds or rodents or other wild life
18	Vermin/ no vermin control
19	Pigs mixed from different ‘sources’
20	Different animal groups (piglets, sows, finishing pigs) kept in one unit (one site production system)
21	Farm with introduction of new animals with quarantine period
22	Farm with introduction of new animals without quarantine period
23	Use of all-in ; all-out management for buildings on the farm
24	Non-use of all in; all out management for building on the farm (continuous throughput)
25	Use of hospital pens, without return of animals
26	Use of hospital pens, with return of animals
27	Other animal species kept on the farm are strictly separated
28	Possibilities of contact of other animal species kept on the farm with the pigs
29	Companion animals kept on the farm are strictly separated
30	Possibilities of contact of companion animals on the farm with the pigs
31	High animal density in pig units
32	High animal density in pig pens
33	Open structure of separation of pens
34	Weaning age of 28 days or more (advice in EU welfare legislation)
35	Weaning age of 21 days or less (not allowed in EU)
36	Temperature inside the units
37	Fresh air enters the stable without filtering
38	Inadequate ventilation
39	Inadequate feed quality
40	Inadequate feed quantity
41	Inadequate heated feed
42	Bore-hole as water source

43	Spreading of human sewage sludge as fertiliser on land around the farm
44	Spreading of manure from the animals on land around the farm
45	Spreading of manure of animals from other (poultry/pig) farms on the land around the farm
46	Exposure to any surface water that may be contaminated with human or animal waste
47	Flooding events that may influence exposure to surface water
48	Use of disinfectants
49	Use of heavy metals
50	Thoroughness of terminal hygiene procedures used to clean empty buildings
51	Floor type
52	Extend of exposure to faeces
53	Feed as a possible source of ESBL
54	Residues of antimicrobial substances in feed
55	Oral administration of antimicrobials
56	Administration of antimicrobials through premix feed
57	Administration of antimicrobials through topdressing feed
58	Administration of antimicrobials through water
59	Administration of antimicrobials through injection
60	Administration of antimicrobials in large groups of animals, > 50% of the stable/unit
61	Administration of antimicrobials in individual animals or small groups, e.g. pen
62	Use of long-acting injectable antimicrobials
63	Inadequate choice of used antimicrobial
64	The more (unnecessary) treatments
65	Too few treatments
66	The number of pigs treated with antimicrobials
67	Dose of used antimicrobial
68	Amino penicillin’s used
69	Use of 3rd and 4th generation cephalosporin’s
70	Use of cephalosporin’s in large groups of animals
71	Use of cephalosporin’s in individual animals
72	Type of cephalosporin (XNL (ceftiofur) / CEQ (cefquinome)…)
73	Use of longacting injectable cephalosporin’s
74	Age of animal treated with cephalosporin’s
75	Number of animals treated with cephalosporin’s
76	Duration treatment with cephalosporin’s
77	Administration of cephalosporin's through feed, injection or water
78	Other types of antimicrobials used (not cephalosporin’s 3rd and 4th gen. or amino penicillin’s)
79	Quantities of other antimicrobials used
80	Co-medication, use of different antibiotics at the same time as a treatment
81	Density of use, how often antimicrobials are used
82	Concentration of the antimicrobial
83	Duration of treatment in which the micro organism is exposed to the antimicrobial, long/prolonged time > 2 weeks
84	Duration of treatment in which the micro organism is exposed to the antimicrobial, too short
85	Species and population sizes of micro organisms present at infection site
86	Adaptability/survivability of ESBL infected strains
87	Age of the animal treated
88	Injuries of the animal (e.g. ear bites)

89	The different diseases targeted by the treatment
90	Adequacy of withdrawal times
91	Animal population resistance to specific ESBL spreading micro organisms
92	Visitors with having animal contact on the farm
93	Employees/visitors with having visited a pig farm in the last 72 hours
94	Employees/visitors entering farm without taking a shower
95	Employees/visitors entering farm without disinfecting hands
96	Employees/visitors entering farm without wearing company clothes
97	Use of same company clothing for the entire farm
98	No separate showers per animal unit/stable
99	No separate disinfection of hands per animal unit/stable
100	Not washing hands after going to the toilet
101	Visitors treated or in contact with antimicrobials otherwise
102	Number of employees
103	Education level of employees
104	Backyard farming employees
105	Employees working on more than one farm
106	Employees visiting pig markets, meetings and shows
107	Family employees working in public health care
108	Farmer or farm workers hospitalised
109	Farmer or farm worker receiving cephalosporin's
110	Farmer of farm worker receiving antimicrobials
111	Illness in farm workers
112	Contact/collective stable with ESBL positive animals now or in the past

Annex 4.

Risk factors for final selection and ranking.

Table 8. Risk factors related to the use of antibiotics

Nr.	Risk factors involved in the use of antibiotics
1	Total amount antibiotics per time unit (per animal unit)
2	Exposure of the intestinal flora
3	The more (unnecessary) treatments
4	Administration of antimicrobials through premix feed
5	Administration of antimicrobials in large groups of animals, > 50% of the stable/unit
6	Dose of used antimicrobial
7	Administration of cephalosporin's through feed, injection or water
8	Use of longacting injectable cephalosporin's
9	Density of use, how often antimicrobials are used
10	Oral administration of antimicrobials
11	Administration of antimicrobials through water
12	Use of cephalosporin's in large groups of animals
13	Use of 3rd and 4th generation cephalosporin's
14	Duration treatment with cephalosporin's
15	Duration of treatment in which the microorganism is exposed to the antimicrobial, long/prolonged time > 2 weeks
16	Quantities of other antimicrobials used
17	Age of animal treated with cephalosporin's
18	Inadequate choice of used antimicrobial
19	The number of pigs treated with antimicrobials
20	Amino penicillin's used
21	Type of cephalosporin (XNL (ceftiofur) / CEQ (cefquinome)…)
22	Use of cephalosporin's in individual animals
23	Other types of antimicrobials used (not cephalosporin's 3rd and 4th gen. or amino penicillin's)
24	Number of animals treated with cephalosporin's
25	Duration of treatment in which the microorganism is exposed to the antimicrobial, too short
26	Age of the animal treated

Table 9. Risk factors related to internal bio-security

Nr.	Risk factors involved in internal bio-security
1	Animal movement within the farm
2	Contact/collective stable with ESBL positive animals now or in the past
3	Spread by employees
4	Education level of employees
5	Thoroughness of terminal hygiene procedures used to clean empty buildings
6	Use of collective instruments and materials
7	Different animal groups (piglets, sows, finishing pigs) kept in one unit (one site production system)
8	Use of hospital pens, with return of animals
9	Extend of exposure to faeces
10	Possibilities of contact of other animal species kept on the farm with the pigs
11	High animal density in pig units

12	Inadequate ventilation
13	Type of farm: Conventional
14	Farm size
15	Weaning age of 21 days or less (not allowed in EU)
16	Use of disinfectants
17	Adaptability/survivability of ESBL infected strains
18	Animal population resistance to specific ESBL spreading microorganisms
19	Use of same company clothing for the entire farm
20	Not washing hands after going to the toilet

Table 10. Risk factors related to external bio-security

Nr.	Risk factors involved in external bio-security
1	Contact/collective stable with ESBL positive animals now or in the past
2	Pigs mixed from different ‘sources’
3	Transport of animals from different farms to the farm
4	Non-use of all in; all out management for building on the farm (continuous throughput)
5	Introduction by people entering the farm
6	Education level of employees
7	Spread by the environment (excl animals
8	Exposure to any surface water that may be contaminated with human or animal waste
9	Feed as a possible source of ESBL
10	Exposure of pigs to wild life
11	Spreading of human sewage sludge as fertilizer on land around the farm
12	Poultry farms within 5 km (3 miles)
13	Transport of animals from one farm to different farms
14	Spreading of manure of animals from other (poultry/pig) farms on the land around the farm
15	Flooding events that may influence exposure to surface water
16	Employees/visitors entering farm without wearing company clothes
17	Use of same company clothing for the entire farm
18	Farmer of farm worker receiving antimicrobials
19	Exposure of feed stores to wild life
20	Spreading of manure from the animals on land around the farm
21	Not washing hands after going to the toilet
22	Employees/visitors entering farm without disinfecting hands
23	Farmer or farm worker receiving cephalosporin’s
24	Farmer or farm workers hospitalized
25	Family employees working in public health care
26	Employees visiting pig markets, meetings and shows
27	Visitors treated or in contact with antimicrobials otherwise

Annex 5.

Ideas to manage risk factors – results of brainstorm

Use of antibiotics

1. Total amount antibiotics per time unit (per animal unit)

The total amount of antibiotics used per time unit and per animal unit is seen as a serious risk factor for the occurrence of ESBL.

1. Taking farm measures to decrease use of antimicrobials, for example different breed of pigs, feed (keeping the intestines healthy) and manage a good climate for the animals
2. Select animal breeds with a higher natural immunity
3. Avoid preventive administration of antimicrobials in large groups of animals but apply individual administration
4. Limit the use of long-acting injectible antimicrobials
5. Limit the use of 3rd and 4th generation cephalosporin's
6. Primarily use of medicines for curative purposes
7. Separating the use of antimicrobials for humans and animals
8. Chart daily dosage per farm per animal category
9. Registration of prescription of antimicrobial per veterinarian
10. Rewarding low antimicrobial use
11. Prevent animal disease in order to use less antimicrobials
12. Adequate fast treatment of diseases and infections
13. Keep the pigs healthier, that will give profit to the farmer
14. Making codes for management that gives insight in the costs of the use of antimicrobials curative and preventive
15. Stimulate the development of vaccines for animal diseases
16. Develop and use alternatives for antimicrobials
17. Aim for an as high as possible 'High Health' / Specific Pathogen Free (SPF) status of the farm
18. Promoting 'High Health'
19. Stimulate study groups that focus on reduction of medicine use
20. Stronger protocol and information from and for veterinarian practices when prescribing antimicrobials
21. Protocols for when antimicrobials can and can not be used
22. Deal with illegal use and import of antimicrobials in the animal husbandry

2. Exposure of the intestinal flora to antibiotics

1. Minimize the use of antimicrobials and other ESBL selection criteria
2. Prevent residues in feed and water
    i. Research after presence of coliforms in water and feed
    ii. Research the presence of resistance of enterobactericae in water and feed
3. Identify selection criteria for ESBL
4. Maintain the balance in intestinal flora (no sudden ration changes)
5. Administration of healthy intestine flora bacteria during and after treatments with antimicrobials

Internal bio-security

Some general ideas on how to manage internal bio-security were given:

1. Heat or chemically disinfect waste products and manure to prevent spread
2. Prevent contact with other animal species

1. Animal movement within the farm

1. Change environmental legislation, to decrease the necessary movement of animals within the farm
2. Adjust the stable system to the growing animal
3. Do not obligate the presence of 'hospital pens' anymore
4. Manage a good carry off of unhealthy animals from the farm
5. Develop a decent cadaver transport from the farm
6. Apply a system that makes it possible to shift animal groups to a new unit without crossing tracks of other age groups, small movements

2. Contact/collective stable with ESBL positive animals now or in the past

1. Application of all in – all out
2. Adequate cleaning of stables
3. Empty stables for a sufficient time duration
4. Monitoring of carriers at the entrance; introduction of ESBL negative animals
5. Separate treated animals from other animals and monitor carriers, make use of 'quarantine' unit

3. Spread by employees and visitors

1. Prevent movements of employees between different units by making (large) units for at least two employees
2. Change clothes and boots, wash hands and disinfect, per unit/ animal category
3. More automation
4. Information and protocol for employees to avoid contact with animals from other farms
5. Separate employees per unit

4. Education level of employees

1. Establish application directed networks, courses and vision evenings
2. Good instruction protocol for (new) employees on hygiene
3. Practice networks in which veterinarian practices, entrepreneurs and chain organizations can exchange successful measures and experiences
4. Discipline and awareness that it will yield something
5. Bringing together knowledge groups of animal diseases, managing more than only ESBL
6. Good communication in the pig sector
7. Consensus about what to communicate

5. Thoroughness of terminal hygiene procedures used to clean empty buildings

1. Evaluate / measure the efficacy of cleaning and disinfection per department
2. Research the efficiency of hydrogen peroxide (H_2O_2) spray in stables (useful in hospitals)
3. Consider when designing pen and unit structures, the possibilities to clean, e.g. no corners
4. Develop solutions for cleaning stables thoroughly
5. Heat treatments of stables, to kill micro organisms

6. Use of collective instruments and materials

1. Use different instrument and materials per animal unit and per animal category
2. Apply self disinfecting materials
3. Move materials together with the animals or use new disinfected material when animals are moved
4. Develop and apply easy applicable disinfection measures
5. Animal units should be large enough to use own material

<u>External bio-security</u>

Next to specific ideas per risk factor, some general ideas on how to manage external bio-security were given:

1. Adequate cleaning of water (drinking water input, waste water output)
2. Vermin control (outside of the farm)
3. Standardizing of diagnostics before animals are entering the farm
4. Create disinfection places along the border for disinfecting all transport entering the country

1. Contact/collective stable with ESBL positive animals now or in the past

Ideas for the management of this risk factor are given under the heading 'internal bio-security'

2. Pigs mixed from different 'sources'

1. Closed system is preferred in which animals go from one farm to one or more other farms. Never from more farms to one farm.
2. A closed system requires a large enough size
3. Also within the farm mixing animal groups should be limited
4. Stimulate farms that separate animal groups
5. Adjust pens to decrease necessary 'mixing' different animals together
6. Separate groups of animals as far as possible to decrease mixing animals

3. Transport of animals from different farms to the farm

1. Closed production chain
2. Fixed contract between different parties (sow farm and fatteners farm)

4. Non-use of all in; all out management for building on the farm (continuous throughput)

1. Apply all in – all out principle
2. In case all in – all out principle is not possible, than minimize it per department or stable
3. Prevent crossing 'walk' and 'load' lines
4. One route, one entrance, one exit (shift system)

5. Introduction by people entering the farm

1. Fixed disinfection protocol
2. Entrance requirements visitors
   a. Do not allow visitors that have visited other pig farms in the last 48 hours
3. Showering
4. Use company clothes
5. As less as possible people into the stables, visitors but also veterinarians and VWA screening employees

6. Limit entrance of people per unit

6. Education level of employees
Ideas for the management of this risk factor are given under the heading ‘internal bio-security’

General ideas
Next to the ideas given above, some ideas were not specific directed to a risk factor or group:

1. Farm
1. Develop stables with bio-security as point of departure
2. The farm size should have a size in which employees can work in separated units to prevent transmission
3. Separate departments clearly, if possible even physically with distance, multi – site principle
4. Increase the distance between farms, especially pig and poultry farms
5. Develop closed buildings with one entrance with hygiene measures
6. When designing a new stable taking into account different aspects like
    a. the limited number of people entering the farm
    b. good possibilities to clean the stable

2. Research
1. Applicable and manageable solutions for the practice in pig farms
2. Find financing for developing quick diagnostics in the animal husbandry sector and identify the risk factors
3. Clarify the link between society and animal husbandry, if there is a link, what is it
4. Research after spread by companion animals
5. Standardizing of diagnostics and treatment and evaluate effects
6. Research after the persistence of ESBL in farms during different stages
7. Improvement of monitoring system is necessary, only a small part of the population coliforms can be ESBL forming, detection depends on the methodology and number of tests

3. Cooperation
1. Establish networks and evaluate, review and improve designs for stables
2. Think together with researchers on new designs and solutions to make them applicable in practice
3. Offer researchers an internship in animal husbandry to give them the opportunity to experience the problems in practice which hopefully can lead to new, good applicable, ideas and solutions

4. Policy
1. Discuss this subject on EU level, a broad approach is needed
2. Place it on the agenda as a ‘social’ problem
3. Be clear in the communication to the public and sector
4. Health program, different statuses in the Netherlands, work to one status
5. A specific status for the Netherlands
6. Create a SPF system like in Denmark
7. Adjust Spatial Planning policy to enable development of SPF farms
8. Finance researches on ESBL and the development of quick diagnostics in animal husbandry