Final report

for the CORE Organic II funded project

"Restrictive use of antibiotics in organic animal farming – a potential for safer, high quality products with less antibiotic resistant bacteria - SafeOrganic"

Period covered: Nov. 2011 – Oct. 2014

Project acronym:	SafeOrganic			
Title:	Restrictive use of antibiotics in organic animal farming – a potential for safer, high quality products with less antibiotic resistant bacteria			
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Start of Project:	1 November 2011	End of project:	31 October 2014	

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Projects website:

The SafeOrganic project is presented at the website of CORE Organic http://coreorganic2.org/safeorganic

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Project summaries, pre- and post/mid-project

Post/mid-term project summary suitable for web publication

- The level of antimicrobial resistance in bacteria from organic vs. conventional pigs has been compared for approximate 25 herds of each production system in DK, FR, IT and SE, by sampling faeces at farm or colon content at slaughterhouse from two pigs per herd.
- An *E. coli* isolate was obtained from each sample and subjected to analysis of MIC (minimum inhibitory concentration) values for comparison of the resistance towards 10 antibiotics between production systems. Resistance to ampicillin, streptomycin, sulphonamides, tetracycline and trimethoprim was most common – ranging from 14% to 75% of *E. coli* isolates from conventional pigs and from 1% to 46% of isolates from organic pigs. The resistance towards these five antibiotics varied markedly between the four countries, but the resistance was always significantly lower in organic pigs than in conventional pigs, except for tetracycline in Sweden, where both production systems had a low occurrence.
- However, DNA based real-time PCR quantification of *cat*, *str*A, *sul*2, *sul*1, *tet*A and *tet*B genes encoding resistances to chloramphenicol, streptomycin, sulphonamides and tetracycline, respectively, was unable to show a difference in the occurrence of resistance genes in the microbiota of organic vs. conventional pigs from the same country.
- The quantitative level of antimicrobial resistance carried by the individual animal in faeces /intestinal content and on the carcass was assessed by determining the number of *E. coli* resistant to tetracycline (TET), out of total *E. coli* (i.e. proportion of TET *E. coli*). Tetracycline is one of the most commonly used antimicrobials in swine production. Except from Sweden, TET resistant *E. coli* was found in close to all pigs of both organic and conventional origin in DK, FR and IT. This was unexpected considering the restrictions on antimicrobial usage in organic pig production. Still, the proportion of *E. coli* carrying TET resistance in the colon was significant lower in the organic pigs, except in Sweden where both production systems had a low occurrence
- Organic pigs are often slaughtered together with conventional pigs implying a risk of contaminating organic pork with resistant bacteria. Therefore, the bacteria present in pigs' own intestinal content was compared with those present on their carcass at end of slaughter to elucidate potential cross-contamination. In France and Denmark, the prevalence of TET resistant *E. coli* was generally lower on the carcasses than in the colon. However, the observed difference in the proportion of TET resistant *E. coli* in the colon of organic vs. conventional pigs was no longer apparent on carcasses. This may indicate that organic pigs are prone to lose their inherent lower level of resistance during the slaughter process.

France is already slaughtering organic pigs before conventional, which points to the importance of hygienic measures in general.

- Different methods were applied in the search for potential markers for (imprudent) antibiotic use in organic animal production. The genetic diversity of bacteria was suggested to be a possible marker as the diversity was hypothesised to be higher in organic pigs due to less selection pressure from antimicrobials. This could however, not be supported as DNA genotyping by PFGE (pulse-field gel electrophoresis) in France revealed a very high genetic diversity of *E. coli* strains in both production systems and it was not possible to associate specific PFGE profiles to either organic or conventional origin. Also an examination of pigs' entire intestinal microbiota by pyrosequencing was unable to resolve a difference between organic and conventional pigs. Alternatively, differences in antibiotic resistance profiles were investigated by PCA (Principal Component Analysis) for the potential as marker. PCA of MIC data for E. coli (IT and SE) was shown to be able to point out organic herds with unusual (conventional-like) resistance profiles. The PCA analysis is seen as a promising tool to enable a more targeted control of antimicrobial consumption in organic pig production.
- Sampling at slaughterhouses is easier and more cost-effective than sampling of live animals at each farm, but foreign bacteria ingested from the surrounding environment (truck, lairage) may bias results obtained at slaughter. Therefore, we investigated for an intestinal sampling site where the level of antibiotic resistance can be considered to resemble the herd status. This was done by assessing the time for ingested particles to reach different intestinal sampling points. The particles were found to reach the mid-colon in 2 of 6 pigs within 4 h. However, the proportion of TET resistant *E. coli* in each pig seemed stable in both caecum and mid-colon independent on sampling time (up to 8 h). This supported that that our sampling procedure at slaughterhouse fitted the purpose of our project.

Pre-project summary

Spread of antibiotic resistance along the food-chain is a major food safety concern due to the risk of treatment failure of human foodborne infections. Recent reports suggest that the restrictions on use of antibiotics in organic animal farming promote lower levels of antibiotic resistance in organic animal products as compared to conventional products. This is, however, scarcely documented in the EU, particularly for swine. Thus, the organic pig production is probably characterized by significant lower levels of antibiotic resistance and providing the documentation of this very important quality parameter of organic pigs holds the opportunity of exploiting this essential advantage of organic pork in marketing. Therefore, in SafeOrganic it will be documented whether the organic pigs in different European countries does show lower levels of antibiotic resistant bacteria compared to the conventional pigs. Furthermore, there seems to be a widespread routine of slaughtering conventional and organic animals at the same slaughter lines without special hygiene barriers to avoid cross-contamination. An important part of the project is therefore to investigate to which degree antibiotic resistant bacteria from conventional raised animals is transferred to organic meat during processing. Accordingly, SafeOrganic will assess and suggest management options, which can minimize the contact and hence risk of cross-contamination between the organic and the conventional meat products during slaughter. Information on the antibiotic use at farm level is normally not available, which hampers the authority control of imprudent use of antibiotics. Therefore, SafeOrganic will investigate if bacterial antibiotic resistance patterns and genotypes can be used as markers for the consumption of antibiotics in organic animal production. The results obtained in the project will be communicated to end-users enabling the slaughter industry to reduce spread of antibiotic resistant bacteria, and organic animal farmers to market pork meat with very low levels of antibiotic resistant bacteria implying an improved food safety quality compared to pork from conventional farming systems.

1. Main results, conclusions and fulfilment of objectives

1.1 Summary of main results and conclusions

The SafeOrganic consortium has held four meetings for planning of activities and discussion of results; kick-off meeting, 21-22 Nov. 2011, Copenhagen; 2nd Annual Meeting 8-9 Nov, 2012, Padova; 3rd Annual Meeting 13-14 Nov. 2013, Uppsala and 4th Annual Meeting 17-18 Sept 2014 Paris. Overall project results were presented at a public Closing Seminar in Copenhagen, 30 Oct. 2014 with approx. 30 external participants. Other dissemination activities incl. student engagements are listed under section 4.

Antibiotic resistance levels in the gastro-intestinal tract of organic and conventional production animals (WP2).

The antimicrobial resistance patterns in organic vs. conventional pigs have been compared by determining the MIC (minimum inhibitory concentration) values for *E. coli* isolates obtained from faeces sampled at farm (IT, SE) or colon content sampled at slaughterhouse (DK, FR, SE). The sampling at slaughterhouse was performed in accordance with the requirements of WP3 to ensure resource efficiency (protocol described under WP3). In France, colon content from 50 pigs from each of 25 organic and 25 conventional herds was collected at one larger slaughterhouse (MIC analysis of 331 *E. coli* isolates). In Denmark, colon content from 52 pigs from each of 26 organic and 26 conventional herds was collected at the only Danish abattoir slaughtering organic pigs (MIC analysis of 104 *E. coli* isolates). Sweden collected colon content from 36 pigs from each of 18 organic and 18 conventional herds at 4 slaughterhouses and in addition faeces from 36 pigs from 18 herds of each production system (MIC analysis of 142 *E. coli* isolates). In Italy, veterinarians have collected faecal samples from 125 pigs from each of 25 organic and 25 conventional herds (MIC analysis of 250 *E. coli* isolates). The comparability of MIC results has been ensured by inter-calibration of the method between partners by participation in the proficiency tests organized by the EU Reference Laboratory - Antimicrobial Resistance (EURL-AR) (D2).

The MIC results showed that resistance to ampicillin, streptomycin, sulphonamides, tetracycline and trimethoprim was most common – ranging from 14% to 75% of *E. coli* isolates from conventional pigs and from 1% to 46% of isolates from organic pigs. The resistance towards these five antibiotics varied between the four countries, but the resistance was always significantly lower in organic pigs than in conventional pigs, except for tetracycline in Sweden, where the resistance was low in general. The resistance to cefotaxime, ciprofloxacin, gentamicin and nalidixic acid was generally low and a difference between organic and conventional pigs was only detectable in Italy. Chloramphenicol was markedly higher in France and Italy compared with Denmark and Sweden and with a significant difference between organic and conventional pigs. As chloramphenicol usage in food animal production is not allowed, it indicates cross-resistance to other antimicrobials. These results are presented in the D2.3 paper.

Also the quantitative level of antimicrobial resistance carried by the individual animal (and on their carcass in WP3) was assessed. To support enough data to allow a comparison between systems and partner countries, this was done by determining the number of *E. coli* resistant to one of the most commonly used antimicrobials in pig production, tetracycline (TET), proportionate to the total number of *E. coli* (i.e. proportion of TET resistant *E. coli*). At the kick-off meeting it was decided to test the appropriateness of 3M™ Petrifilm™ Select *E. coli* count plates for enumeration of the proportion of *E. coli* resistant to TET. This method was adapted from Wu et al. (2008). An in-house validation was performed by each partner with *E. coli* strains with known MIC (minimum inhibitory concentration) values, and Petrifilm containing 64 mg/L TET was shown to provide a satisfactory differentiation between resistant and susceptible strains (D2).

Except in Sweden, TET resistant *E. coli* was found in nearly all pigs of both organic and conventional origin in DK, FR and IT, which was unexpected considering the restrictions on antimicrobial usage in organic pig production. Still, the proportion of *E. coli* carrying TET

resistance in the colon was significant lower in the organic pigs, except in Sweden where both production systems had a low occurrence (Paper D2.3).

For defining the herd status on antibiotic reistance, sampling at slaughterhouses will be a much easier and more cost-effective than sampling of live animals at each farm. However, sampling of faecal material at slaughter may potentially be biased by foreign bacteria ingested from the environment during transport and lairage. This may lead to misclassification (bias) of the antimicrobial resistance status of the herd. To avoid this, we investigated for an intestinal location where faeces could be considered “herd like”. We did this by assessing the time for ingested inert particles fed at the farm prior to delivery to reach different intestinal sampling points (caeceum and mid-colon). The particles were found to reach the rectum within 8 hours. For easy identification of the sampling point we focused on the mid-point of the colon spiral. Even though the particles reached the mid-colon in 2 of 6 pigs already within 4 h after intake, the proportion of TET resistant *E. coli* in each pig seemed to be quite stable in both caecum and mid-colon independent on sampling time (up to 8 h). Even though ingested foreign bacteria may have reached the sampling point we concluded that the overall level of resistance (proportion of TET resistant *E. coli*) and the likelihood of picking a resistant *E. coli* isolate for MIC testing would not significantly differ between samples taken at the herd and samples taken at slaughter. This supported that sampling at slaughterhouse for the purpose of our project was an appropriate alternative to sampling in the herd (results presented in D2.2).

In addition to the core activity on *E. coli* across all partners, each pig in France and Sweden was tested for *Campylobacter* and in France also for *Salmonella.* In France, the *Campylobacter* prevalence in colon content was high in both organic (76.8%, n=56) and conventional pigs (74.0%, n=58). The resistance towards eight antibiotics was tested and the percentage of isolates resistant to streptomycin was high in both production systems. Otherwise, the resistance towards nalidixic acid, ciprofloxacin, erythromycin and tetracycline was significantly lower in organic pigs compared with conventional pigs.

In Sweden**,** *Campylobacter coli* was isolated in colon content from 66.7% (n=36) and 55.6% (n=36) of conventional and organic pigs respectively. The prevalence in fecal samples (collected at farm) was of the same magnitude, 44.4% (n=36) and 61.1% (n=36) in conventional and organic pigs respectively. A total of 82 isolates of *Campylobacter coli*, 40 isolates from conventional and 42 from organic pigs, were obtained from the 36 colon and 36 fecal samples. Resistance to six antibiotics was tested. The highest percentage of resistance was also in Sweden to streptomycin, 48% and 64% in isolates from conventional and organic pigs, respectively. Resistance to nalidixic acid and ciprofloxacin was lower (30-40%) and there was no difference between production systems. Only two isolates, both from conventional pigs, were resistant to tetracycline while no isolate was resistant to erythromycin or gentamicin.

In France *Salmonella* analysis was performed on a total of 113 samples of colon content and 114 carcass swab samples from 26 organic and 31 conventional herds. The resulting *Salmonella* prevalence in colon content was 37.9% in organic and 32.7% in conventional pigs (*P*=0.563) and on carcasses 10.7% and 10.3% respectively. *Salmonella* was most often found resistant to streptomycin and tetracycline followed by ampicillin and chloramphenicol and with no significant difference between organic and conventional. No *Salmonella* was resistant to cefotaxime, ciprofloxacin, colistin, gentamicin, meropenem, nalidixic acid or trimethoprim/sulfonamide. The results on *Campylobacter* and *Salmonella* will be presented in two scientific papers in preparation.

The organic production systems for pork meat in partner countries were described at the kick-off meeting in Copenhagen as background for planning the survey in T2.3. Information on herd characteristics and slaughter logistics was collected in relation to the survey, which revealed difficulties in collecting sufficient data across countries of high enough uniformity for specific risk factor analysis on the full data set. Additionally, in Italy and Sweden, organic and conventional

slaughter pig herds were asked about their consumption of antimicrobials (AB) showing that group treatment was predominant in Italy (usage in fattening period) while individual treatment was more common in Sweden. Denmark extracted data on the AB consumption for each participating herd via the Danish database VETSTAT. However, it appeared to be difficult to obtain comparable data across countries in terms of method of AB administration, units as well as length and timing of administration. Despite this, significant efforts were made to perform risk factor analysis of sub-sets of data (e.g. country wise). For the occurrence of TET resistant *E. coli* by MIC, these analyses pointed to country, type of production system, season and TET consumption as important factors.

Contamination of organic products with resistant bacteria from conventional products during processing (WP3)

For assessment of the potential cross-contamination of organic pigs with antimicrobial resistant bacteria from conventional pigs during slaughter (DK, FR, and SE) a common laboratory protocol for sampling and bacteriological analyses was established by France to ensure comparability of results between partners. The optimal sampling scheme was agreed to be sampling of the last conventional pig herd before changing to slaughter of organic herds (if feasible at the involved slaughterhouses). The first organic herd was then sampled thereafter. From each herd the first and last animal were sampled. The pigs identified by this scheme were each sampled twice along the slaughter chain: 1) colon content at evisceration (also used for WP2.3) and 2) a carcass swab prior to cooling.

The occurrence of TET resistant *E. coli* was tested on 104, 100 and 72 carcasses in DK, FR and SE, respectively. In Sweden, TET resistant *E. coli* was rare in both production systems. In France and Denmark, the prevalence of TET resistant *E. coli* was generally lower on the carcasses than in the colon. However, the observed difference in the proportion of TET resistant *E. coli* in the colon of organic vs. conventional pigs was no longer apparent on carcasses. This may indicate that organic pigs are prone to lose their inherent lower level of resistance during the slaughter process. These results are presented in the D3.1 paper.

In France, the occurrence of TET resistant *E. coli* positive carcasses was observed to increase from 48% for the first 200 pigs slaughtered to 63% for the 200-500th pig and 69% for the last pigs after the 501thpig. This support our hypothesis that cross-contamination may build up in the slaughter environment during the day implying a higher contamination risk for pigs being slaughtered in the end of day. However, since French slaughterhouses always slaughter organic pigs first in the week, cross-contamination from conventional pigs to organic pigs is here very unlikely.

In France, the efficacy of Pulsed-Field-Gel-Electrophoresis (PFGE) for studying cross-contamination between organic and conventional pigs could be assessed. DNA genotyping of a total of 614 *E. coli* isolates obtained from colon and carcass. The PFGE analysis revealed a very high diversity of PFGE profiles, which hindered a distinguishing between isolates of different origin, In parallel, PFGE genotyping was also performed on 104 *Salmonella* and 240 *Campylobacter*, but as for *E. coli* the diversity was also high for these species and combined with relative few isolates from carcass the PFGE typing was deemed unable to resolve potential cross-contamination.

Identification of potential markers for antibiotic use in organic animal production (WP4)

For investigation of the correlation between the observed AR in swine herds and antimicrobial consumption the general study design was inadequate to support this. Instead the Italian farm survey and in part the Swedish survey was designed to provide the sufficient number of bacterial isolates per farm, which would be necessary for the data analysis. The aim of this part of the study was to develop a tool for public control which can identify farms with a potential

imprudent use of antimicrobials. PCA (Principal Component Analysis) was chosen as the statistical method to analyse MIC data on the occurrence of antimicrobial resistance in *E. coli* isolates from conventional and organic pig farms in Sweden and Italy. The PCA analysis was shown to group selected herds according to their antimicrobial resistance profile. Thus, the analysis was able to describe if an organic herd displayed a conventional-like resistance pattern. The tool has the advantage that it can point out herds independent of the quality of the information on antibiotic use provided by the farmer. In cases of imprudent use the quality of the information from the farmer may be questionable. These results are presented in the D4.1 paper.

The restricted use of antibiotics in organic farming was hypothesized to lead to a larger diversity in the intestinal bacterial flora in organic pigs compared to conventional pigs. Potential differences in genotype diversity were assessed by PFGE typing (using X*ba1* enzyme) of French *E. coli, Salmonella* (using X*ba1* enzyme) and *Campylobacter* (using *Kpn1* enzyme) isolates from colon contents. A total of 193 *E. coli* strains from organic pigs (n=101) and conventional pigs (n=92) showed a high diversity of 164 PFGE profiles with 82 of organic as well as conventional origin. No profile was shared between the two pig production systems and within each production system there was also no shared profile between herds.
A total of 80 *Salmonella* strains from organic pigs (n=44) and conventional pigs (n=36) were distributed into 12 PFGE profiles with 9 different profiles among organic strains and 8 different profiles among conventional strains. Five profiles were shared between organic and conventional pigs. For *Campylobacter*, the 240 typable strains resulted in a high diversity of 122 PFGE profiles with only 1 PFGE profile shared between organic and conventional pigs.
The large diversity in the PFGE genotyping observed in both production systems did thus not support the hypothesis that organic management practices with restrictions on antimicrobial consumptions and access to open areas should result in a larger genetic diversity.

Microbial diversity between organic and conventional pigs was approached also by culture independent analysis of the microbiota in colon content and faeces from pigs from the four countries, Denmark (n=96), France (n=78), Italy (n=248) and Sweden (n=71). First, the total load of selected antimicrobial resistance genes was determined. Total DNA was purified from the 493 faecal samples and subjected to real-time PCR quantification of *cat*, *str*A, *sul*2, *sul*1, *tet*A and *tet*B genes encoding resistances to chloramphenicol, streptomycin, sulphonamides and tetracycline, respectively. Unexpectedly, there were no significant differences between the prevalence of the selected antibiotic resistance genes in organic and conventional pigs collected in the same country. However, differences between countries were observed as samples originating from southern countries exhibited significantly higher antibiotic resistance gene abundance than those from northern part of Europe. The selected resistance genes represented some of the most common resistances found by MIC in both production systems. While the MIC results of E. coli showed significant differences between organic and conventional for these antimicrobials the analysis of the total microbiota by the rt-PCR method could not resolve this. The latter may be due to the crudeness of the latter method.
In parallel, the purified DNA was also used for comparing the composition of the intestinal microbiota in organic vs. conventional pigs by pyrosequencing the V3/V4 regions of 16S rRNA genes. By this method, it was not possible to reveal any significant differences in the overall microbiota composition across all the tested samples. These results are presented in the D4.3 paper.

As an alternative to display microbial diversity, the genome of a total of 200 *E. coli* isolates from organic and conventional pigs raised in Sweden, Italy, France and Denmark have been whole genome sequenced. The still ongoing substantial bioinformatic analysis aims to establish if *E. coli* isolates from organic pigs possess specific and unique genetic sequences and properties as compared to *E. coli* from conventional pigs. Comparison analysis between the four countries will also be done. This will be reported within the frame of SafeOrganic.

1.2 Fulfillment of objectives

The survey of the antimicrobial resistance level in organic pigs compared with conventional pigs proofed to show that the resistance in *E. coli* bacteria was lower in organic pigs in DK, FR and IT, while Sweden had low levels of resistance in both production systems. This was both showed by MIC testing of single isolates and quantitatively by calculating the proportion of TET resistant *E. coli* within each animal.

Colon content and carcass swabs were sampled from the same pig, at slaughterhouses processing pigs both of organic and conventional origin in DK, FR and SE, but it was difficult to fully elucidate the possible cross-contamination of organic carcasses with resistant bacteria. However, the observed difference in the proportion of TET resistant *E. coli* in colon of organic and conventional pigs (DK, FR) was no longer apparent on their carcass, which indicated a potential cross-contamination between pigs. This was observed also in France alhough France slaughtered the organic pigs before conventional, probably indicating a general need for hygienic precautions during slaughter.

Italian principal component analysis of MIC profiles of faeces samples from conventional and organic pigs was able to point out non-typical organic AR profiles. This can help to do more targeted control of antimicrobial consumption in organic pig production. The PFGE genotyping revealed a very high diversity in strains (*E. coli*, *Salmonella* and *Campylobacter*) and it was not possible to associate specific PFGE profiles to organic or conventional origin by this method. As an alternative, sequencing of a total of 200 *E. coli* isolates has been undertaken, but awaits data analysis.

The project results were presented at a final seminar with approx. 30 external participants representing veterinarians, organic farming organisations, agricultural branch organisations, journalists, private companies and universities, and the seminar resulted in min. two news articles. This has put the attention to the benefits of organic pig production in terms of lower AR levels, but also the potential problems on maintaining low AR levels during slaughter.

2. Milestones and deliverables status

Milestones:

No[1]	Milestone name	Planned delivery month[2]	Actual delivery month[2]	Means of verification
M 1.1	Kickoff meeting	1	1	Meeting minutes
M 1.2	Establishment of advisory committee	2	3	List of members
M 2.1	An inter-calibration of MIC determination between partner laboratories	6	6	Concordance between results from partner laboratories.
M 2.2	Survey on AR level - collection of faeces samples from pigs of organic and conventional origin	18	24	Survey complete and bacterial strain collection established
M 3.1	Survey on cross-contamination - collection of intestinal content/carcass swabs from org./conv. pigs at slaughter line	18	24	Survey complete and bacterial strain collection established

Deliverables:

No[1]	Deliverable name and language	Nature[3]	Dissemination level[4] and link to the document	Planned delivery month[2]	Actual delivery month[2]
D 1.1	Annual meetings	Meeting	INT	1-36	1-36
D 1.3	Final workshop	Workshop	PU/RE	36	36
D 2	Inter-calibration of methods	Protocols	INT	4	4
D 2.1	Selection and description of organic and conventional pig herds for survey of antibiotic resistance (AR) level	Protocol	INT	6	6
D 2.2	Comparison of sampling in herds and abattoirs	Paper	PU	18	36*
D 2.3	Comparison of antibiotic resistance levels in organic and conventional pigs	Paper	PU	24	36*
D 3.1	Cross-contamination of organic pigs with antibiotic resistant bacteria during slaughter	Paper	PU	27	36*

[1] Please use the numbering convention <WP number>.<number of milestone/deliverable within that WP>. For example, deliverable 4.2 would be the second deliverable from work package 4.
[2] Measured in months from the project start date (month 1).
[3] Please indicate the nature of the deliverable. For example Report, Paper, Book, Protocol, Prototype, Website, Database, Demonstrator, Meeting, Workshop…
[4] Please indicate the dissemination level using one of the following codes: PU = Public; INT= Internal (Restricted to other project participants); RE = Restricted to a group specified by the consortium; CO = Confidential, only for members of the consortium.

D 4.1	Assessment of antibiotic patterns as potential predictor of antibiotic consumption in organic animal production	Paper	PU	33	36*
D 4.2	Assessment of differences in genotype diversity in organic vs. conventional pigs	Paper	PU	36	36*
D 4.3	Assessment of differences in AR genes and microbiotia in organic vs. conventional pigs	Paper	PU	30	36*

Additional comments (in case of major changes or deviation from the original list)

The milestones associated with the survey on the antimicrobial resistance level in organic vs. conventional pigs as well as cross-contamination (WP2 and WP3) were postponed up to 6 months. Based on discussions at the kick-off meeting it was decided that sampling should be performed equally around a calendar year for the results to take into account seasonal variations, which was found sound considering the relative small delay this would cause. *For all the deliverables involving scientific papers, the papers have been drafted. However, data analysis and interpretation has been more challenging than expected, especially for D2.2 and D3.1. The results for D4.2 based on PFGE typing have been presented in abstracts and oral presentations and an alternative whole genome sequencing approach have been initiated but await final analysis. *The papers will be finalized in the coming months by the responsible partners.

3. Work package description and results:

WP 1	**"Title" Project management and communication**
Responsible partner: "partner no, institution acronym and name of WP manager" **No. 1, DTU FOOD, Søren Aabo**	
Original description of work: T1.1 *Management, reporting and accounting*. Planning of project activities and exchange of results between the partners will be done via a kickoff meeting, at annual meetings and on meetings on demand. A Steering Group comprising the project coordinator, WP-leaders and selected key persons will at least every six months discuss the progress of the project at the annual meetings or via Telephone or videolink-meetings. Each WP-leader is responsible for reporting the entire WP-activity and results to the Project Coordinator who will do overall reporting to the European Commission. T1.2 *Advisory Committee and networks.* A four-five member Advisory Committee representing key stakeholders (e.g. representatives from organic animal producers, the slaughter industry and consumer organizations) and leading experts within the field of research will be formed. The committee will particularly be consulted on the planning and means of communication of research activities and will be invited to the annual meetings. A transnational SafeOrganic network will be established and supported by the gathering of people to meetings and by establishing a common project webpage. Further, exchange of post–docs, PhD, and MSc students within the project will be an important part of the network activity. T1.3 *Internal and external communication*. Internal: Info concerning the T1.1 activities, such as meeting agenda/minutes will be communicated between project participants by email from the Coordinator or by upload to a project website (limited access). External: The established Advisory Committee will support contact to networks within organic animal farming and it is considered to have one of the annual	

meetings in Brussels to promote contact to decision and policy makers in the European Commission. Project results will be presented at conferences and in popular as well as peer-reviewed publications. A final workshop will be arranged for all project participants and invited guests from authorities, representatives for organic animal farmers, slaughter industry, and retailers for discussion and dissemination of results. Other EU member states, which are providing money into the CoreOrganic II will specifically be invited.

Report on results obtained and changes to the original plan/WP aims:
A- results obtained:

T1.1 *Management, reporting and accounting*.

In addition to e-mail communication between partners, the following meetings has been held for planning of activities and discussion of results; kick-off meeting, 21-22 Nov. 2011, Copenhagen; 2nd Annual Meeting 8-9 Nov, 2012, Padova; 3rd Annual Meeting 13-14 Nov. 2013, Uppsala and 4th Annual Meeting 17-18 Sept 2014 Paris. Extended summaries with decisions from the meetings have been distributed to the SafeOrganic partners.

T1.2 *Advisory Committee and networks.*

The Advisory Committee has been established with the following members: Simme Eriksen, Denmark; Anna Maria Baraldi, Italy; Anna Wallenbeck, Sweden; Armelle Prunier, France. The advisory had been invited to the annual meetings.

T1.3 *Internal and external communication*.

In addition to e-mail exchange of meeting agendas, protocols, etc., a shared DropBox folder has been established (partner access only) for upload/storage of project documents. Master/bachelor students have been associated with the project for the performance of their experimental work. The project and preliminary results have been presented at CORE Organic meetings, national/international conferences and finalized results will be also be presented at coming conferences. The projects partners presented the main project results at the Closing Seminar in Copenhagen, 30 Oct. 2014 with approx. 30 external participants and the seminar resulted in two news articles.

B- comments on deviations from the original plan:

WP 2	**"Title" Antibiotic resistance levels in the gastro-intestinal tract of organic and conventional production animals**

Responsible partner: "partner no, institution acronym and name of WP manager"
No. 4, SVA, Björn Bengtsson

Original description of work:

T2.1 *Animal production systems and antibiotic use practices*.

The organic production systems for pork meat in partner countries will be described. Selected representative organic and conventional slaughter pig herds (IT, SE) will be characterized with regards to factors, e.g. herd management, mortality, use of disinfectants to ensure hygienic production facilities and use of antibiotics (e.g. based on consumption databases when available), that potentially are associated with the development of antibiotic resistance. DK will provide data from other studies in this task.

T2.2 *Assessment of more convenient methods for faecal sampling.*

In task 2.3, the bacteriological status of the pigs at herd level will be characterized by sampling of colon content from pigs at slaughter. Since the intestinal microbiota may change during transport and lairage, a bias may be introduced to these results obtained at slaughter. Any such bias will be quantified in task 2.2 (DK) and if relevant an adjustment for it will be suggested. From each of 20-30 individual animals, originating from different farms, 10 colon isolates of *E. coli* per animal will be obtained from the same animal at the farm and at slaughter. The subtype diversity will be compared between the farm sample and the slaughterhouse sample by molecular methods.

T2.3 *Comparison of the occurrence of antibiotic resistance in conventional pigs and organic*

pigs.

Antibiotic resistance levels will be determined in pigs from selected organic and conventional animal farms by susceptibility testing of the faecal indicator bacteria *E. coli* (all partners), *Enterococcus faecium* (optional) and of *Salmonella* (FR) and *Campylobacter* (FR, SE). Based on the practical conditions among the different partners, faecal samples will be taken either from individual animals at the farm or from intestinal content (colon) at the slaughterhouse. Faecal samples will be collected from two animals from each of 25 organic and 25 conventional herds. Dilution series of the faecal samples will be cultured on selective media. Two isolates of relevant bacterial species per sample will be tested for antibiotic resistance according to the scheme below. The established collection of bacterial isolates comprising up to 100 isolates per bacterial species for each production system (organic and conventional) and for each partner country provides the basis for WP4.

We also investigate if pigs from organic farms carry less resistant bacteria in the intestine than pigs from conventional herds. For this purpose, total colony material will be harvested from agar plates containing 200-300 *E. coli* colonies for testing of the proportion of resistant *E. coli* by parallel plating on non-selective media and media containing antibiotic. The choice of antibiotics will depend on what is found most relevant for each partner according to the test results or existing data.

International recognized standard methods for determination of minimum inhibitory concentrations (MIC) by microdilution (e.g. by EUCAST and CLS) will be used for susceptibility testing by all partners. Antibiotics to be studied (Table 1) are those recommended for monitoring of resistance in *E. coli*, *Salmonella* and *Campylobacter* by EFSA (EFSA Journal. 2007, 2008). Epidemiological cut-off values issued by EUCAST will be used for categorization of isolates as susceptible or resistant. Comparable test results between partners will be ensured by inter-calibration tests on a defined strain collection.

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:

T2.1 *Animal production systems and antibiotic use practices.*

The organic production systems for pork meat in partner countries were described at the kick-off meeting in Copenhagen as background for planning the survey in T2.3. Information on herd characteristics and slaughter logistics was collected in relation to the survey, which revealed the difficulties in collecting sufficient data across countries of high enough uniformity for specific risk factor analysis. Additionally, in Italy and Sweden, organic and conventional slaughter pig herds were questioned about their consumption of antimicrobials (AB) showing that group treatment was predominant in Italy (usage in fattening period) while individual treatment was more common in Sweden. Denmark extracted data on the AB consumption for each participating herd via the Danish database VETSTAT. However, it was difficult to obtain comparable data across countries in terms of method of AB administration, units as well as length and timing of administration. Nevertheless, significant efforts were made to perform risk factor analysis of sub-sets of data (e.g. country wise). For the occurrence of TET resistant *E. coli* by MIC, these analyses pointed to country, type of production system, season and TET consumption as important factors.

T2.2 *Assessment of more convenient methods for faecal sampling.*

An examination of the intestinal location of inert particles fed at the farm at delivery to slaughter (DK) served as a proxy for ingested bacteria during transport and lairage. It was shown that the particles, and thus potentially also bacteria ingested by the pig after leaving the farm, can reach the mid-colon of pigs within 4 h after intake. Despite this, the proportion of antimicrobial resistant *E. coli* seemed unaffected at any of the intestinal sampling sites investigated (caecum and mid-colon) independent on sampling time (up to 8 h). Indicating that sampling at slaughterhouse for this purpose is appropriate. A protocol for sampling of mid-colon content (WP2.3 and WP3.1) was established and the scientific paper is in preparation (D2.2)

T2.3 *Comparison of the occurrence of antibiotic resistance in conventional and organic pigs.*

The antimicrobial resistance patterns in organic vs. conventional pigs has been compared by determining the MIC (minimum inhibitory concentration) values for *E. coli* isolates obtained from faeces sampled at farm (IT n=25; SE n=18) or colon content sampled at slaughterhouse (DK

n=26; FR n=25; SE n=18). The number of herds sampled of each production system is given by the *n,* and two pigs were sampled from each herd except in Italy, where five pigs were sampled from each herd in relation to WP4.
The MIC results showed that resistance to ampicillin, streptomycin, sulphonamides, tetracycline and trimethoprim was most common – ranging from 14% to 75% of *E. coli* isolates from conventional pigs and from 1% to 46% of isolates from organic pigs. The resistance towards these five antibiotics varied between the four countries, but the resistance was always significantly lower in organic pigs than in conventional pigs, except for tetracycline in Sweden, where the resistance was low in general. The resistance to cefotaxime, ciprofloxacin, gentamicin and nalidixic acid was generally low and a difference between organic and conventional pigs was only found in Italy. Chloramphenicol was markedly higher in France and Italy compared with Denmark and Sweden and with a significant difference between organic and conventional pigs.
The quantitative level of antimicrobial resistance carried by the individual animal or on their carcass was assessed by determining the number of *E. coli* resistant to one of the most commonly used antimicrobials in pig production, tetracycline (TET), compared with the total number of *E. coli* (i.e. proportion of TET resistant *E. coli*). Except in Sweden, TET resistant *E. coli* was found in nearly all pigs of both organic and conventional origin in DK, FR and IT, which was unexpected considering the restrictions on antimicrobial usage in organic pig production. Still, the proportion of *E. coli* carrying TET resistance in the colon was significant lower in the organic pigs, except in Sweden where both production systems had a low occurrence
Additional analysis for *Campylobacter* in France showed a high prevalence in both production systems. Resistance testing towards eight antimicrobials showed that the percentage of isolates resistant to streptomycin was high in both production systems. Otherwise, the resistance towards nalidixic acid, ciprofloxacin, erythromycin and tetracycline was significantly lower in organic pigs compared with conventional pigs.
In Sweden, the prevalence of *Campylobacter coli* was relative high in both conventional and organic pigs 44-67%. Resistance testing towards six antibiotics showed also a high resistance to streptomycin in Sweden, while nalidixic acid and ciprofloxacin resistance was lower (30-40%) but without difference between production systems. Only two isolates, both from conventional pigs, were resistant to tetracycline.
Also analysis of *Salmonella* in France showed an equal prevalence in organic and conventional pigs. *Salmonella* was most often resistant to streptomycin and tetracycline followed by ampicillin and chloramphenicol and with no significant difference between organic and conventional.

B- comments on deviations from the original plan:
In relation to solving task 2.2., it was discussed at the kick-off meeting that the suggested genotyping of a large number of *E. coli* isolates may not be able to indicate appearance of foreign bacteria ingested by the pig during transport and lairage. Therefore it was decided to assess the intestinal passage time of ingested pellets as a proxy of contamination encountered in the surrounding environment, supplemented with an assessment of the stability of the proportions of TET resistant *E. coli* in different sampling sites.
In the survey comparing antimicrobial resistance between partner countries, it has not been possible to cover the same sampling period nor to sample over a whole year in all the involved countries as planned during the kick-off meeting in Copenhagen. This was due to practical obstacles for the personnel performing the sampling and for the different slaughter houses involved. Furthermore, the proposal described MIC analysis of two isolates per faecal sample in relation to WP2.3, this was changed to one isolate from faeces and one from carcass, which would better help to support the cross-contamination task in WP3 without further costs. Finally, the 3M™ Petrifilm™ Select *E. coli* count plates for enumeration of the proportion of *E. coli* was implemented as convenient measure for the level of resistance.

WP 3	"Title" Contamination of organic products with resistant bacteria from conventional products during processing

Responsible partner: "partner no, institution acronym and name of WP manager"
No. 3. ANSES, Martine Denis

Original description of work:

T3.1 *Transfer of antibiotic resistant bacteria from conventional raised animals to organic meat when slaughtered together at the same processing line.*

We hypothesize that conventional meat contaminates organic meat when slaughtered together, leading to a more conventional-like occurrence of antibiotic resistance on organic carcasses. We will investigate the level of cross-contamination occurring on the slaughter line by analysing for an increase in the proportion of antibiotic resistant strains on the carcasses of organic pigs compared to the proportion found in their intestinal content. The considered bacteria will be *E. coli* (all partners), *E. faecium* (optional) and *Salmonella* (FR) and *Campylobacter* (FR, SE).

The proportion of antibiotic resistant bacteria will be measured by comparing direct plate counts from faecal samples and carcass swabs (before chilling) from the same animal using non-selective and selective media containing the relevant antibiotic (based on WP2). Samples from 25 animals from organic farms and 25 pigs from conventional farms will be tested. The level of agreement between the proportion found on organic carcasses and conventional pigs will indicate the level of cross-contamination.

As an alternative measure of transfer, we will investigate the potential of using changes in resistance patterns or genotype diversity (e.g. by pulsed-field-gel-electrophoresis, PFGE profiling). This will be done on samples from selected carcasses with indication of a high level of cross-contamination.

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:

For assessment of the potential cross-contamination of organic pigs with antimicrobial resistant bacteria from conventional pigs during slaughter (DK, FR, and SE) a common laboratory protocol for sampling and bacteriological analyses was established by France to ensure comparability of results between partners. The optimal sampling scheme was decided to be sampling of the last conventional pig herd (the first and last animal in the batch was sampled) before slaughtering of organic herds (if feasible at the involved slaughterhouses). The organic herd preceeding immediately after this conventional pig herd was then sampled (again the first and last animal from this herd were sampled). The pigs identified by this scheme were each sampled twice along the slaughter chain: 1) colon content at evisceration (also used for WP2.3) and 2) a carcass swab prior to cooling.

The occurrence of TET resistant E. coli was tested on 104, 100 and 72 carcasses in DK, FR and SE, respectively. In Sweden, only very few *E. coli* was obtained from carcasses and TET resistant *E. coli* was rare in both production systems. In France and Denmark, the prevalence of TET resistant E. coli was generally lower on the carcasses than in the colon. However, the observed difference in the proportion of TET resistant *E. coli* in the colon of organic vs. conventional pigs was no longer apparent on carcasses. This may indicate that organic pigs are prone to lose their inherent lower level of resistance during the slaughter process.

In France, the potential cross-contamination of organic carcasses with (resistant) bacteria from conventional pigs was assessed by Pulsed-Field-Gel-Electrophoresis (PFGE) DNA genotyping (using *Xba*1 enzyme) of 374 E. coli isolates from colon and 240 isolates from carcass. The 614 isolates resulted in 435 different profiles indicating a very high diversity. Common profiles for colon content and carcasses were observed for individual pigs but also between different pigs. Although the later suggests cross-contamination, these pigs were slaughtered on separate days so the current PFGE genotyping did not confirm transfer of bacteria between the two pig productions.

Additional analysis of 104 *Salmonella* isolates in France yielded 7 serovars and 16 different PFGE profiles of which 7 (84% of the strains) were common between organic and conventional pigs. Still, only one *S.* Typhimurium PFGE profile was common in organic and conventional carcasses during the same sampling day. Moreover, with only 20 isolates from 12 carcasses it was unfeasible to make conclusions on the potential *Salmonella* cross-contamination between

organic and conventional pigs along the slaughter chain.
Extended results on occurrence, resistance and genetic diversity of *E. coli, Salmonella* and *Campylobacter* have been presented orally and/or in abstracts and posters (see dissemination list under 4).

B- comments on deviations from the original plan:
Three partners participated in the WP3 (FR, DK and SE) while slaughterhouse sampling was unfeasible in Italy. For Italy, this allowed the project to spend more resources for the testing at farm level related to WP4. In France, it was not possible to sample organic pigs after conventional as they always are slaughtered first in the morning. Also, successive herds of the same production system were sampled the same day in order to reduce the number of sampling days for the 25 herds.
The considered bacteria are *E. coli* (FR, DK and DE), *Salmonella* (FR) and *Campylobacter* (FR, SE). Testing for *E. faecium* was optional but was abandoned due to complexity of the detection and numeration protocols.
The PFGE genotyping of numerous *E. coli* and *Salmonella* isolated in France revealed that the diversity of PFGE profiles does not enable conclusions on the cross-contamination potential. Therefore, extended data analysis has been performed and is still ongoing in an attempt to address the cross-contamination issue, which has proven more difficult than anticipated. Not least due to very few *E. coli* positive carcasses in Sweden, and the French deviation from the optimal sampling plan – organic pigs after conventional – for assessment of cross-contamination.

WP 4	**"Title" Identification of potential markers for antibiotic use in organic animal production**

Responsible partner: "partner no, institution acronym and name of WP manager"
No. 2, IZSVe, Antonia Ricci

Original description of work:
T4.1 *Correlation between antibiotic resistance patterns and antibiotic consumption.*
A possible correlation between the observed antibiotic resistance pattern and the consumption of antibiotics at herd level will be investigated using appropriate statistical tools (FR, IT, DK). In practice, we will relate antibiotic resistance patterns from Danish organic and conventional herds to data on antibiotic consumption, registered in the Danish Vet-Stat database (DK). Thereby we aim to establish a code that translates an observed resistance pattern in a herd into an estimate of the actual consumption of antibiotics in the herd. This translation matrix will be applied to estimate the antibiotic consumption in organic and conventional herds in partner countries without any registration of consumption (FR, IT, SE) and a classification of farms into different levels of consumption will be attempted. Moreover, such tool may be useful in public control to reveal imprudent antibiotic consumption in organic farming.

T4.2 *Differences in the genotype diversity in bacteria from organic and conventional animals*.
Higher genotype diversity within bacterial indicator species (e.g. *E. coli or Enterococci*) or pathogens (e.g. *Salmonella* and *Campylobacter*) from organic animals is expected due to the presumable lower antibiotic selection pressure. We investigate if the genotype diversity can be used to differentiate between organic and conventional pork. The genetic diversity of bacterial strains isolated from organic and conventional production systems in WP2 and WP3 will be determined by DNA based typing methods (e.g. RFLP/PFGE). Relevant harmonised typing protocols for these bacteria (e.g. PulseNet and CDC) will be applied and strains will be discriminated according to their similarity (Struelens et al. 1996).

T4.3 *Culture independent quantification of microbiota and antibiotic resistance genes in organic and conventional animals*
DNA extracted from faecal samples of WP2 by the same commercially available kit (all partners) will be subjected to quantitative RT PCRs (Sybr Green format). The RT PCRs will be targeted to

known antibiotic resistance genes and to variable parts of 16S rRNA genes for specific taxons, respectively. The antibiotic resistance specific PCR will probably target the *strA* and *sul2* genes, which exhibited a high prevalence in pig faecal samples and high variability when pigs from different farms were tested (I. Rychlik, unpublished data). The taxon specific PCRs will be targeted to detect the orders *Bifidobacteriales*, *Lactobacillales*, *Clostridiales*, *Bacteroidales*, *Enterobacteriales* and *Verrucomicrobiales*, and phylum *Fusobacteria*. The cycle threshold (Ct) values of genes of interests will be normalised to Ct values of PCR using universal 16S rRNA primers detecting the presence of bacterial DNA. In this way, the relative proportion of e.g. *Lactobacillales* out of all bacteria present in faeces, or the proportion of *strA* encoding bacteria out of all bacteria will be determined and correlated with the anamnestic data of the sample.

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:

T4.1 *Correlation between antibiotic resistance patterns and antibiotic consumption.*

IZSVe researchers visited DTU Food for exploration of the usefulness and feasibility to collect and relate DANMAP and VETSTAT data, in order to fulfil the T4.1 objective. Due to the encountered difficulties, the opportunity to use data from a Danish project named QualySafe was considered. However, during the second project meeting, important differences among the countries in terms of antimicrobial resistance patterns were highlighted, which impaired the use of the Danish VETSTAT and QualySafe data. Therefore it was agreed to use data raised from the project activities, and in particular data from farm sampling (i.e. IT and SE), as input for the statistical model. The aim was to develop a tool for identification of farms according to their potential probability of using antimicrobials in an imprudent way. This was done by using MIC data on the occurrence of antimicrobial resistance in *E. coli* isolates from conventional and organic pig farms in Sweden and Italy.

PCA (Principal Component Analysis) was chosen as statistical method for discriminating the sampled farms in terms of antimicrobial resistance patterns. Results demonstrated that PCA allowed discrimination of selected herds according to their antimicrobial resistance profile. Thus, the developed tool for classification of farms, according to their presumptive use of antimicrobials seemed to fit the purpose.

T4.2 *Differences in the genotype diversity in bacteria from organic and conventional animals*.

Potential differences in the genotype of bacteria from organic and conventional pigs were assessed by PFGE typing (using X*ba1* enzyme) of French *E. coli, Salmonella* (using X*ba1* enzyme) and *Campylobacter* (using *Kpn1* enzyme) isolates from colon contents. The diversity of PFGE profiles was very high as the majority of isolates carried its own PFGE profile. Accordingly, it will not be possible to associate bacterial strains to their origin, organic or conventional production, on the basis of their PFGE profiles.

As an alternative, the full genome of a total of 200 *E. coli* isolates from organic and conventional pigs raised in Sweden, Italy, France and Denmark have been sequenced. Ongoing DNA sequence analysis aims to establish if *E. coli* isolates from organic pigs possess specific and unique genetic sequences and properties as compared to *E. coli* from conventional pigs. Comparison analysis between the four countries will also be done.

T4.3 *Culture independent quantification of microbiota and antibiotic resistance genes in organic and conventional animals*

Culture independent quantification of antimicrobial resistance genes in the microbiota from pigs of organic and conventional origin were performed on colon content/faeces from four countries, Denmark (n=96), France (n=78), Italy (n=248) and Sweden (n=71). Total DNA was purified from the 493 faecal samples and subjected to real-time PCR quantification of *cat*, *str*A, *sul*2, *sul*1, *tet*A and *tet*B genes encoding resistances to chloramphenicol, streptomycin, sulphonamides and tetracycline, respectively. Unexpectedly, there were no significant differences between the prevalence of the selected antibiotic resistance genes in organic and conventional pigs collected in the same country. However, there was a country effect as samples originating from southern countries exhibited significantly higher antibiotic resistance gene abundance than those from northern part of Europe. The selected resistance genes represented some of the most common resistances found by MIC in both production systems. While the MIC results of *E. coli* showed significant differences between organic and conventional for these antimicrobials

the analysis of the total microbiota by the rt-PCR method could not resolve this.
The purified DNA was also used for comparing the composition of the intestinal microbiota in organic vs. conventional pigs by pyrosequencing the V3/V4 regions of 16S rRNA genes. By this method, it was not possible to reveal any significant differences in the overall microbiota composition across all the tested samples.

B- comments on deviations from the original plan:
In the search for a correlation between AR patterns and antimicrobial consumption it was found too difficult to explore the existing data in VETSTAT as more than one bacterial isolate per herd would be required. Instead the Italian farm survey was designed to provide more isolates per farm for data analysis.
In relation to task 4.2, the results of the PFGE genotyping revealed a too high diversity of PFGE profiles to resolve potential genetic differences between microbiota in organic vs. conventional pigs. Therefore, this task was at a late stage supplemented by full genome sequencing of 200 isolates of *E. coli*, which still awaits data analysis, but will be finalized in the beginning of 2015.
In T4.3, the analysis of the intestinal microbiota was performed by pyrosequencing the V3/V4 regions of 16S rRNA genes, which can reveal all major taxonomic groups down at family level. The originally planned rt-PCR can only reveal specific targeted groups of known bacteria and provide much less information compared with sequencing, and as sequencing has now become a cost-effective alternative to rt-PCR it was applied for this task.

4. Publications and dissemination activities

4.1 List extracted from Organic Eprints

Project description
Aabo, S. (2011) SafeOrganic. CORE Organic Research Seminar, Paris, France, 29 November 2011.

Aabo, S. (2011) SafeOrganic. Presentation at: Statusmøde Organic RDD og CORE Organic II, Horsens, Denmark, 16 November 2011.

SafeOrganic: *Restrictive use of antibiotics in organic animal farming – a potential for safer, high quality products with less antibiotic resistant bacteria.* Runs 2011 - 2014. Project Leader(s): Søren Aabo, Research Leader, Phd. DVM, National Food Institute, Technical University of Denmark.

Project leaflets:
Anonymous. (2012) Antibiotic usage in organic pigs - will consumers benefit from restricted antibiotic usage in organic pigs? (2012).

Jensen, AN. and Aabo, S. (2014) SafeOrganic - Restrictive use of antibiotics in organic animal farming – a potential for safer, high quality products with less antibiotic resistant bacteria (2014).

Conference paper, poster, etc.
Jensen, AN, Thanou, O, Axelsdottir, A, Thomsen, SG and Aabo, S (2012). Evaluation of intestinal sampling sites in pigs at slaughter for assessing antibiotic resistance level in swine herds. Abstract and Poster at: 23rd International ICFMH Symposium, Food Micro 2012, Istanbul, Turkey, 3-6 Sept. 2012.

Kérouanton A, Chidaine B, Rose V, Kempf I & Denis M (2014) *Campylobacter* in organic and conventional pig productions in France: occurrence, antibiotic resistance and genetic diversity. Food Micro 2014 Nantes, France, 1-4 september 2014 (Proceeding Abstract p462).

Kérouanton A, Chidaine B, Rose V, Kempf I and Denis M (2014) *Campylobacter* chez les porcs biologiques et conventionnels : prévalence et antibiorésistance. 10ème congrès de la Société

Française de Microbiologie -SFM, Paris, 31 March – 1 April 2014 (Proceeding Abstract no C024, p26).

Kérouanton, A, Rose, V, Chidaine, B, Kempf, I and Denis, M (2013). Comparison of organic and conventional pig productions on prevalence, antibiotic resistance and genetic diversity of *Escherichia coli.* Oral presentation. 9th International Symposium On Epidemiology and Control Of Foodborne Pathogens In Pork - SafePork, 9-12 september, 2013, Portland, Maine, USA (Proceeding Abstract no. 1605).

Kérouanton A, Rose V, Chidaine B, Kempf I and Denis M (2014). Résistance à la tétracycline et diversité génétique *d'Escherichia coli* isolés de porcs biologiques et de porcs conventionnels. *Journées de la Recherche Porcine* - JRP2014, Paris, France, 4-5 February 2014 (Proceeding Abstract no S11, pp179-180).

Kérouanton, A, Rose, V, Even, M, Houard, E, and Denis, M (2013). Prevalence and genetic diversity of *Salmonella* in organic and conventional pig productions in France. *I3S,* International Symposium Salmonella and Salmonelosis (I3S), May 27-29, 2013, Saint-Malo, France. (Proceeding Abstract no. 65162, pp 279-280 and Poster).

Kérouanton A, Rose V, Houard E and Denis M (2013). Occurrence and genetic diversity of *Salmonella* in organic and conventional pig productions in France. 10th International Meeting on Microbial Epidemiological Markers - IMMEM 10*,* 2-5 October, 2013, Institut Pasteur, Paris, France (Proceeding Abstract p98).

Newspaper and magazine title

Aabo, S and Jensen, AN (2013) Restricted use of antibiotics in organic pig farming. *ICROFS News*, March 2013, 1, p. 11.

Jensen, AN. and Aabo, S. (2014). Slagterier giver bakterier til økosvin. Landbrugsavisen 12 Dec. 2014.

Nielsen, KM. (2014). Økologi halverer resistens. Økologi og Erhverv, 555, 14 Nov. 2014

4.2 Additional dissemination activities

Oral presentations

Dalsgaard, A and AN Jensen. (2012) Presentation at: Statusmøde Organic RDD og CORE Organic II, Årslev, Denmark,13 Dec. 2012.

Kérouanton A, Rose V, Chidaine B, Even M and Denis M (2012). Diversité des *E.coli* chez les porcs biologiques et porcs conventionnels. Journée d'information et d'échanges en filière porcine, Anses Ploufragan, France, 20 Novembre 2012.

Kérouanton A, Chidaine B, Rose V, Kempf I and Denis M (2014) *Campylobacter* chez les porcs biologiques et conventionnels : prévalence et antibiorésistance. 10ème congrès de la Société Française de Microbiologie -SFM, Paris, 31 March – 1 April 2014 (Proceeding Abstract no C024, p26).

Kérouanton A, Chidaine B, Rose V and Denis M (2014). *Campylobacter et Salmonella chez les porcs biologiques et conventionnels: prévalence, caractérisation phénotypique et génotypique*. Journée filière porc, ANSES, Ploufragan, France, 11 Dec. 2014.

Österberg, J. "SafeOrganic, an EU-project on antibiotic resistance in conventional and organic pigs" - WP 2.3 results during 2014. Presented
1) at "SVA Projektråd" (20 invited stakeholders from national authorities and organizations)
2) for local pig farmers collaborating with the Swedish University of Agricultural Sciences

Master thesis: Olga Thanou. Title: Monitoring of the tetracycline resistance level in *Escherichia coli* from farm to slaughter. Department of Veterinary Disease Biology, University of Copenhagen

Report – Student special course: Axelsdottir, Aslaug; Thomsen, Signe Gerling. June 2012. *Enterobacteriaceae* in pigs. National Food Institute, DTU, Denmark

Posters

Jensen et al. 2012 (same as above) presented at Dansk Mikrobiologisk Symposium, 5 Nov. 2012, Copenhagen, Denmark

Thanou, O., A. Axelsdottir, S.G. Thomsen, A.N. Jensen and S. Aabo. 2012. Evaluation of intestinal sampling sites in pigs at slaughter for assessing antibiotic resistance level in herds. Poster at 10th Symposium on Food Microbiology, Helsingør, Denmark, 9-10 May 2012,

Österberg J, Jensen AN, Cibin V, Denis M and Bengtsson B (2014). Resistance to tetracycline in *Escherichia coli* from pigs in organic and conventional production in four European countries, 6th European Symposium on Porcine Health Management, Sorrento, Italy, 7-9 May 2014 (Proceeding Abstract no P27, p144).

Papers in prep.

(D2.2) Jensen et al. Robustness of faecal samples obtained during slaughter for determination of antibiotic resistance in swine herds

(D2.3) Österberg et al. Antibiotic resistance in *Escherichia coli* from pigs in organic and conventional farming in four European countries

(D3.1) Denis *et al.* Antibiotic resistance in *E. coli* on organic and conventional pig carcasses at slaughter in three European countries: assessment of cross-contamination from conventional to organic pigs during process

(D4.1) Cibin et al. Antimicrobial resistance patterns as predictors of pig farms exposure to antibiotics

(D4.2) Kérouanton *et al*. Occurrence, antimicrobial resistance and genetic characterization of *Salmonella* isolated from organic and conventional pigs in France

(D4.2) Kérouanton et al. *Campylobacter* in organic and conventional pig productions in France and Sweden: occurrence, antibiotic resistance and genetic diversity

(D4.3) Gerzova et al. Characterization of antibiotic resistance gene abundance and microbiota composition in faeces of organic and conventional pigs from four EU countries

4.3 Further possible actions for dissemination

- List publications/deliverables arising from your project that Funding Bodies should consider disseminating (e.g. to reach a broader audience)

- Indicate publications/deliverables that could usefully be translated (if this has not been done, and indicate target language).

As some results are still immature further dissemination awaits further progress of analysis.

4.4 Specific questions regarding dissemination and publications

- Is the project website up-to-date?

The presentation of SafeOrganic project at the website of CORE Organic http://coreorganic2.org/safeorganic is updated annually.

- List the categories of end-users/main users of the research results and how they have been addressed/will be addressed by dissemination activities

The dissemination activities have targeted the scientific community within the area of microbiology by participation in conferences incl. engagement of students as well as presentation of the project results to the organic community at meetings and in newsletters/leaflets. The projects partners presented the main project results at the Closing Seminar in Copenhagen, 30 Oct. 2014 (D1.3) with approx. 30 external participants representing veterinarians, organic farming organisations, agricultural branch organisations, journalists, private companies and universities. The seminar resulted in at least two news articles.

- Impact of the project in relation to main beneficiaries of the project results
  (Note: for the different categories of end-users/main users of the research results, explain how well the project has been able to reach these target groups, and any known impact)

In SafeOrganic, we have shown that organic pigs in different European countries (DK, FR and IT) have lower levels of antimicrobial resistant *E. coli* compared to the conventional pigs, while Sweden had low levels of resistance in general. This potentially allows organic pig producers to market this as an added value of improved food safety for organic pork products compared with conventional. Also, further analysis of the main drivers for the low AR levels in both production systems in Sweden may provide valuable info about how to lower the spread of AR in pig production in general. However, slaughterhouse investigations indicated that the observed difference in proportions of TET resistant *E. coli* in colon between organic and conventional pigs was not maintained on the carcasses. As France already is slaughtering organic pigs before the conventional, other hygienic measures seem necessary to maintain the low AR level. Dissemination of the project results has put attention to the potential need for initiatives to avoid contamination of organic pork with resistance, although the exact mechanisms behind the spread are uncertain.
Finally, it appeared difficult to identify markers for the consumption of antimicrobials in organic animal production. Nevertheless, principal component analysis of the MIC profiles of faeces samples (IT and SE) successfully allowed to point out organic pigs with non-typical AR profiles. This will be communicated to authorities as a more targeted control option for imprudent use of antimicrobials, particularly where no registration of consumption is in place.

5. Added value of the transnational cooperation in relation to the subject

As the project results have shown, the antimicrobial resistance status can differ significantly between countries and regions, and data obtained at national level incl. suggestions for control options may therefore not be adoptable by EU in general. The current survey including four countries will help to take into account the different levels of antimicrobial resistance scenarios existing within the EU. The observed low level of antimicrobial resistance in Sweden in both organic and conventional pig production may serve as an example on how to achieve lower resistance in pig production in general. In this context it is noteworthy that the consumption of antimicrobials was registered to be none for organic herds in Italy, and still the level of resistance was quite high. This emphasizes the complexity of the mechanisms behind spread of resistance. It will require a more in-depth analysis for identifying the most significant management practices responsible for driving the development of resistance. Difficulties in aligning data between countries due to quite different conditions for organic pig production and slaughter among the participating countries limited the performance of a proper risk factor analysis across countries. Nevertheless, the scrutinizing of data can help to identify knowledge gaps and challenges in future research addressing control of spread of antimicrobial resistance in pig production.

The SafeOrganic addressed the level of antimicrobial resistance in pigs mainly on basis of *E. coli*. However, the recent increased focus on spread of methicillin resistant *Staphylococcus aureus* (MRSA) in pigs makes it relevant to ask whether organic pig production serve beneficiary in fighting the spread of MRSA.