Danish Research Centre for Organic Farming

Final Report

For DARCOF II research projects financed by grants from
The Directorate for Food, Fisheries and Agro Business
under the Danish Ministry of Food, Agriculture and Fisheries

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1. Research program

Research in organic farming 2000-2005 (DARCOF II)

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2. Project title and number

Bacterial infection risk associated with outdoor organic pig production

- **with special reference to *Salmonella* and *Campylobacter* infection**
- **SaCaFree FØJO II project no. II.10**

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3. Head of project

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Slutrapporten sendes elektronisk til Forskningscenter for Økologisk Jordbrug *foejo@agrsci.dk* senest 3 måneder efter projektets afslutning.

Slutrapporten vedlægges et dansk resumé.

5. Other project staff

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6. Project period (month, year)

Start of project: 1st July 2002
End of project: 30th June 2004

7. Final report

A. Project summary

The modern consumers are becoming increasingly critical of the quality of meat and other types of products of animal origin. Focus is put on the animal husbandry and the way of production concerning animal welfare. This leads to increasing interest for organic, free-range or other kinds of animal-friendly production systems where the animals benefit from a low animal density and good possibilities for expressing normal behaviour. In general, consumers also expect products from these kinds of systems to be of a higher microbiological quality compared to products from conventional production systems. However, today there is no documentation for a lower level of the most common zoonotic bacterial infections (e.g. *Salmonella*, *Campylobacter* and *Yersinia*) in organic or other alternative production systems.

The objective of this project was to improve the knowledge on the risk of outdoor pig production in relation to spread and persistence of *Campylobacter* and *Salmonella* infections. For *Salmonella* the specific objectives were to evaluate the survival of *Salmonella* Typhimurium in soil and grass of contaminated pastures used for outdoor pig production, measurement of the infectivity of naturally *S*. Typhimurium contaminated pastures in relation to time. For thermophilic *Campylobacter*, the objectives were to describe the infection dynamics of natural *Campylobacter* infections over time in outdoor pigs, including time of colonisation, level of excretion in faeces, species distribution in the group and in the individuals, interaction with the environment, and to describe the possible changes in prevalence and species distribution in relation to time and environmental contamination.

Experimental pastures for production of outdoor organic piglets were sat up in 2003. Experimentally infected piglets were grouped together with uninfected tracer piglets in order to monitor the transmission of infection. In addition, soil samples was analysed to determine the level of contamination. After a period of 6 weeks the pastures were vacated. A new set of uninfected piglets was introduced in each pasture to monitor if these animals become infected due to their habitation in the *Salmonella* contaminated pastures. The pastures and animals were sampled and monitored by bacteriological (qualitatively and semi-quantitatively) and by serological examinations. Due to a large variation of infection dynamics in the first two periods among "identical" pastures, an investigation of the pathogen-reducing effect of ploughing in the third period was omitted. Based on the results in the second period, the study on the infectivity of the contaminated pastures continued in one pasture with introduction of new salmonella-free tracer-animals, whereas the set-up from the first period with artificially infection of pigs was repeated in the remaining three pastures, where there was no or little contamination left in the environment.

A method was developed and evaluated for determining the species composition (*C. jejuni* and *C. coli*) in faecal samples and subsequent isolation of the species in minority. The groups of pigs that served as controls in the experimental *Salmonella* infection study was also used for studying the dynamics of natural *Campylobacter* infection in outdoor pigs. The piglets were monitored for natural colonisation of thermophilic *Campylobacter* species, and the ratio between *C. jejuni* and *C. coli*. Likewise, environmental samples were analysed throughout the experimental periods. Isolates from animals and environment have been identified to the species level and serotyped to monitor the dynamics of the infection: number of different strains in each pig and persistence/exchange of strains over time.

The results will be available for the scientific community through publication in reviewed journals

and communicated to organic farmers through general journals. The large amount of quantitative data obtained in this project provides useful information for use in the risk assessment of zoonotic infection in organic pig production.

Table A.1: Work package list (from application)

No.	Work package title	Participants*	Budget (1.000 DKK)	Start	End	Deliverable no(s):
1	**Investigation of introduction and spread of *Salmonella* in outdoor pigs**	Dorte Lau Baggesen Eva M. Nielsen, Annette N. Jensen	1,325	2002	2004	1-6
2	**Investigation of the dynamiscs of natural *Campylobacter* infection in outdoor pigs**	Eva Møller Nielsen, Dorte L. Baggesen, Annette N. Jensen	675	2002	2004	7-11

* Responsible participants are underlined

Objectives and expected achievements

The overall objective of the project is to improve the knowledge on the dynamics behind environmental spread of two important zoonotic bacteria – *Salmonella* and *Campylobacter* – within outdoor pig production systems.

Good management procedures – good farming practice – based on scientific evaluation of the risks of introduction and persistence of zoonotic infections is essential for the redevelopment of the organic production. The present proposal focuses on studies to describe the dynamics of zoonotic infections in outdoors pigs and evaluate the risk of outdoor production in relation to spread and persistence of *Campylobacter* and *Salmonella* infections in pigs. This include

- evaluation of the survival of *Salmonella* Typhimurium in soil and grass of contaminated weaning and fattening pastures for outdoor pig production systems
- measurement of the infectivity of naturally *S.* Typhimurium contaminated pastures in relation to time, and
- in the case of high infectivity, evaluation of the pathogen reducing effect of soil treatment
- description of the prevalence of natural campylobacter infection over time in outdoor pigs including description of the species distribution in the group and in the individuals
- evaluation of the possible changes in prevalence and Campylobacter species distribution in relation to time and environmental contamination

Through the described project new important knowledge regarding salmonella and campylobacter infections in outdoor pig production systems will be obtained. This includes information on the infectivity of *Salmonella* under natural conditions, e.g. on the risk for healthy piglets to be infected from infected animals or contaminated environment. The investigations will provide quantitative information on the infection and contamination levels, which is presently very limited. In the case of a very high contamination level in the pastures the study will provide additional information on the pathogen reducing effect of the ploughing pastures. In addition, new information on the infection dynamics of thermophilic campylobacters in outdoor pigs is expected especially in relation to the level of colonisation, distribution of different species and types, the possible co-infection of *C coli* and *C. jejuni*, and the transmission between animals and the environ-

ment.

The achievements obtained will be formulated in practical guidelines directly applicable for the organic pig producers in order to minimize the risk of zoonotic infection in organic pig herds. In addition, results will be available for the scientific community through publication in reviewed journals. The large amount of quantitative data obtained in this project will deliver the necessary information for use in quantitative risk assessment of zoonotic infection in organic pig production. This achievement will be strength by the interaction with other projects carried out by the present group (Wildlife as a source of salmonella infection in food-animal production, FØSI00-SVS-6, which has been extended by *Campylobacter* investigations**;** "Grundlag for rådgivning vedr. foderets betydning for salmonellainfektion i økologiske svinebesætninger" [The significance of feeding in relation to salmonella infections in organic pig herds] , J.nr. 93S-2462-Å01-00981).

C. Progress and results

C.1 Description (summary) of main results and conclusions

Detection of salmonella

The optimal method for detection of salmonella in faecal and soil samples was assessed by comparison of the standard bacteriological culturing method and a molecular real-time PCR method previously applied for identification of salmonella presumptive isolates. First, a method for preparation of a salmonella culture with a known number of cells for spiking of non-infected sample material was established, which could be valuable also for future evaluations of detection limits of different methods. Then, three different strains of *Salmonella*, *S*. Typhimurium DT 12, *S*. Typhimurium DT 12 rifampicin resistant and *S*. Dublin were evaluated with pre-enrichment of ca. 5 CFU per 25 g sample. The pre-enrichment step was performed in parallel in conventional buffered peptone buffer (BPW) and BPW added Novobiocin (22 mg/L), which previously has been shown to increase the number of salmonella after pre-enrichment. This was also confirmed in this study, as the best overall detection of *Salmonella* was obtained with the bacteriological method using pre-enrichment in BPW containing Novobiocin. Detection of *Salmonella* by rt-PCR was about similar but not better than the culturing method and therefore not beneficial. Moreover, the current rt-PCR would not have been able to differentiate the inoculum strain from other *Salmonella* serotypes, which would still require bacteriological culturing methods. Thus, the bacteriological detection method with pre-enrichment in BPW with Novobiocin was applied throughout the experimental study.

Experimental set-up - transmission of salmonella in pigs

Experimental outdoor pastures were sat up at Rørrendegård (research animal facility under the Danish Royal Veterinary and Agricultural University) on a 3-year-old grass/clover field not previously grazed. A total of 6 pastures, with four pastures for 10 pigs each (50 m^2 per pig) and two pastures for 8 pigs each, were made according to the requirements for organic pig production. A total of 3 times 56 organic pigs, 7-8 weeks of age (post weaning) were purchased from an organic pig farmer, to ensure that the study results would take in any potential benefits of the organic rearing, with respect to the susceptibility to salmonella infections. All pigs were tested salmonella-negative before initiation of the experiments.

In the first period of the study, 3 of the 10 pigs in each of the 4 pastures were given 10^7 *S*. Typhimurium DT 12 rifampicin resistant cells (oral) or 10^9 cells at day 1 in an attempt to establish 2 low and 2 highly contaminated pastures. The contamination level was adjusted by number of ingested cells instead of number of infected animals, to obtain a similar number of tracer animals in each pasture. Examinations of faeces samples 3 days after inoculation of the pigs indicated the difficulties in the establishment of an artificial infection of *Salmonella,* as some inoculated pigs appeared salmonella-negative and only few inoculated pigs remained salmonella-

positive all 6 weeks. However, the level of salmonella was sufficient to cause salmonella infections in some of the salmonella-free tracer-pigs. More than half of the tracer-pigs were tested salmonella-positive at least once during the 6 weeks period, but persistent salmonella infections were mainly seen in one of the pastures, where a high inoculation dose had been applied. The semi-quantitative culturing method indicated a rather low salmonella excretion level in most pigs <100 CFU/g.

Due to the results obtained within the first weeks of monitoring, it was decided to terminate the first period after 6 weeks instead of the proposed 2 months. This was in order to maintain a significant contamination level in the environment for examination of the infectivity of a naturally contaminated pasture in the second period. For such study it was considered important to introduce the new salmonella-free pigs immediately after removal of the first-period pigs. Since it was not practical feasible to remove and process all 56 pigs for post-mortem examinations within one day, these examinations were omitted. In a few cases, animals were excluded from the experiment due to health problems and these animals were subjected to post-mortem examinations to state if salmonella caused their poor condition. One pig died due to salmonellosis (inoculated pig) and another two killed pigs had salmonella in liver and spleen.

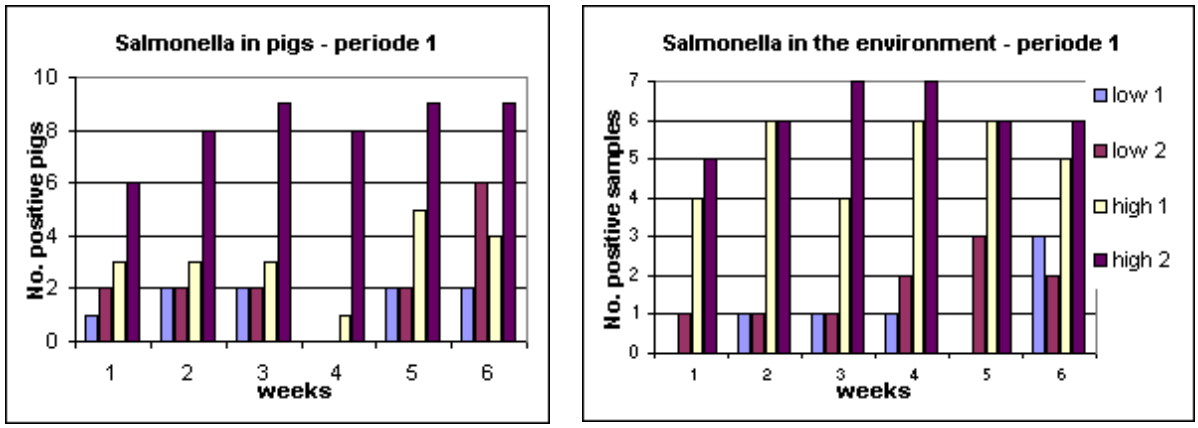
Infectivity of the environment. The naturally contaminated pastures lead to infection of the introduced salmonella-free tracer-pigs (2nd period) in mainly one pasture, whereas none or few pigs became infected in the other pastures. The high infection rate in this pasture (high dose) coincides with the pig with clinical symptoms of salmonellosis in the 1st period that excreted a high level of salmonella before it died. This indicates the importance of eliminating pigs with signs of salmonella infections as soon as possible, as these animals probably contribute significantly to the persistence of infections. Nevertheless, the second period also showed that the susceptibility to infections varies between individual pigs, since two pigs were found salmonella-positive although little salmonella was detected in the environmental samples, while none was found salmonella-positive in a pasture (high dosis) where almost half of the environmental samples were positive throughout the 6 weeks.

Third period. The set-up for the third experimental period was determined by the outcome of the second period. Since mainly one of the contaminated pastures gave rise to infections in the second period, an evaluation of the pathogen-reducing effect of ploughing in comparable pastures was not feasible. Introduction of new salmonella-free tracer-pigs in the continuously contaminated pasture resulted in 2 salmonella-positive pigs at 3 occasions, although the salmonella inoculum strain from the first period persisted in the pasture throughout the whole experiment.
In the other 3 pastures, a very low contamination level was found at the end of the second period. Thus, the set-up from the first period was repeated, but with the high dose of salmonella cells only. To differentiate between salmonella used for new inoculations from salmonella potentially surviving from the first two periods, the inoculum strain was further selected for nalidixic acid resistance. Again, a big variation in the infection susceptibility was found, as not all of the inoculated pigs were salmonella-positive 3 days after inoculation, while there still was a transmission to some of the salmonella-free tracer-pigs.

In general, the study showed that it was a bit difficult to control the artificial contamination level by the oral inoculation, as the pigs responded differently. However, the model with inoculation of 3 animals in each pasture ensured that none of the pastures were salmonella-negative, allowing us to study the transmission of salmonella to salmonella-free pigs. Choosing two different doses of cell also helped us to avoid a situation, where the tracer-pigs were either all positive or all negative, which would have given less nuanced information on the infection dynamics. Individual pigs showed a big variation in the susceptibility to salmonella infections. Further examinations of which host factors account for these differences in pigs of same age and background, would be helpful in the understanding and thus prevention of salmonella infections.

Survival of salmonella in the non-host environment.
Once a week, 1 water and 6 surface soil samples were colleted from the pasture environment at different locations and tested bacteriological (qualitative) for *Salmonella*. The salmonella inoculum-strain was detected in the pasture environment in all but one pasture at the first sampling. Furthermore, most salmonella-positive samples were found in pastures where a high inoculum dose had been applied. No salmonella was applied in the second period so the detected salmonella must have survived from the first period in the non-host environment or by re-ingestion into the new pigs. Pigs in two pastures remained salmonella-negative throughout the second period, while salmonella was still detected for up to 6 weeks in the pasture environment.

The high number of salmonella-positive environmental samples continued in the third period and therefore the non-host survival of salmonella was followed for 7 weeks after the removal of the pigs. Salmonella was detected in soil for up to 5 weeks and in huts until last week (7) of examination. This indicates the importance of good hygiene measures to prevent persistence of salmonella infections.



Figur 1: Number of salmonella excreting pigs and level of salmonella contamination in each pastures (1st. periode)

Faeces contamination of the environment.
The contamination load in the pasture was also followed through a common faeces indicator organism, *E. coli,* in order to supplement the salmonella data, especially in case it was difficult to recover any salmonella from such non-host environment. Thus, a quantitatively method for enumeration of presumptive *E. coli* in soil and water samples were established, based on selective media and biochemical reactions. These studies showed that a faeces contamination was build up throughout the pasture environment (7 locations) and the examinations until 7 weeks after removal of pigs indicated the rate of decline. The *E. coli* data provided quantitative data on the faeces load, which supplemented the qualitative data on salmonella.

Introduction of Salmonella.
In addition to the inoculum strain, *S.* Typhimurium DT 12 rifampicin/naldixic resistant, an unexpected high number of different serotypes and *S.* Typhimurium phagetypes were found in pig faecal and soil samples, and occasionally these were also found in the control pastures. To try to elucidate the source of these types, rodents and birds was caught in the surrounding environment for a period of 2 weeks. This was performed in collaboration with the Danish Pest Infestation Laboratory. However, no *Salmonella* was found, whereas *Campylobacter* was found especially in the crow birds. These results have been presented at a seminar at Wageningen University and have been accepted for publication in Wageningen Journal of Life Sciences (in press).
During the experiment, cases of PMWS were experienced among the pigs and some pigs of poor condition were killed. This may have increased the susceptibility of the pigs and partly explain the unexpected high colonisation of different *Salmonella* bacteria besides the inoculum strain.

Identification and detection of Campylobacter

Real-time PCR method. An in-house real-time PCR method for discrimination of the four Campylobacter species *C. upsaliensis, C. lari, C. coli* and *C. jejuni* has been further tested for specificity against Camp. spp and other closely related organisms. This assay was further developed for screening of enriched pig faecal samples for mixed infections of *C. jejuni* and *C. coli*. For this purpose, two different enrichment broths, Preston and Bolton without blood and in addition two sample preparation methods, boil lysate and chelex were tested. Bolton enrichment broth without blood and boil lysate method were chosen and allowed detection of *C. jejuni* in pig faecal samples. The ability to hydrolyse hippurate is the only phenotypic characteristic that distinguishes *C. jejuni* from *C. coli*. But this bacterial isolate identification method is often problematic, as false hippurate-negative *C. jejuni* can be misidentified as *C. coli*. Thus, the developed molecular rt-PCR method is a very helpful tool for identification and discrimination between these two species.

Specific detection of C. jejuni colonies. The rt-PCR screening enhanced the detection of mixed infections in pig faecal samples, however, it is still valuable to obtain bacterial isolates for further characterization of the strain type diversity. A colony-blot hybridization method, for detection of *C. jejuni* colonies present in minority among other *Camp.* spp. on mCCD agar plates, was developed and evaluated. First a DIG-labelled *C. jejuni* species-specific nucleotide probe, based on the hippuricase gene of *C. jejuni* was developed. The hybridization of this probe to DNA from *C. jejuni* colonies blotted onto a nylon membrane allowed detection of *C. jejuni* colonies and following isolation of bacterial strains from these colonies.
C. coli is predominant in pigs, which is contrary to most other animals were *C. jejuni* is dominant. The developed method enhanced the specific detection of *C. jejuni*, which may go undetected by the conventional methods for isolation and identification of *C. jejuni* from pigs. These methods have also been used in an in-house study on conventional pigs. The evaluation of the rt-PCR and colony-blot hybridization methods with 74 reference strains (*Campylobacter* spp. and closely related organisms) and a comparison of the usability of the different methods for detection of *C. jejuni* in pig faeces have been submitted as a scientific paper.

Campylobacter infections in pigs.
In all three periods the control pigs were examined from 8 to 14 weeks of age for excretion level of *Campylobacter* spp., which fluctuated with no clear tendency over time. In addition to the specific detection of *C. jejuni,* five *Camp.* ssp. were isolated randomly from each pig and environmental samples every second week. All the obtained isolates (ca. 1000) were stored as a freeze collection for further analysis. The specific detection of *C. jejuni* with real-time PCR screening of pre-enriched faecal samples and colony hybridisation showed the campylobacter species distribution in the organic pigs, often with *C. jejuni* in 100-fold lower numbers than *C. coli*. In the first two periods, only a few pigs were found to host *C. jejuni*, whereas *C. jejuni* was found at least once in 12 of 15 pigs in the third period, but still in minority to *C. coli*. These preliminary results were presented (oral) at SAFE PORK 2003, The 5th International Symposium on the Epidemiology and Control of Foodborne Pathogens in Pork.
It was suggested that outdoor pigs perhaps would show a higher prevalence of *C. jejuni* compared to indoor pigs due to an increased exposure to *C. jejuni* in wild animals. However, the rather different *C. jejuni* detection rate between the 3 experimental periods did not allow any clear conclusion on this. Furthermore, our 'prevalence' data was not directly comparable with data base results from conventional pigs obtained with the conventional methods.
Interactions with the environment. Rt-PCR identification of the obtained campylobacter isolates showed that *C. coli* was predominant in pigs and also in the environmental samples (ca. 80% of isolates), whereas *C. jejuni* only was detected in crow-birds and rats. Serotyping of the isolates will further indicate the possible interaction between the pigs and the environment. This work has

been initiated but is not fully completed. All the results on the campylobacter infection dynamics will be presented in a scientific paper.

All together, the project has improved our knowledge of infection dynamic of zoonotic infection in outdoors organic pig production significantly. The overall conclusion is in relation to salmonella that even though the infectivity seems limited under these production conditions, server problems can occur under unfavourable circumstances. In this study, high infection pressure with high risk of persistence and spread of infection to other individuals was established in one of seven experiments. This implies a big infection risk for the slaughter pig and thereby risk of contamination of the final products. Contamination with zoonotic bacteria as salmonella and campylobacter reduces the integrity of the final product in relation to consumer preference and confidence, which in the end can lead to minimised sale.

It is, therefore essential to identify the factors that influence the sensitivity and/or the infection resistance of the individual pigs, as this study indicates that the large variation among pigs may be influenced by host factors. A project with focus on infection resistance against bacterial infection in organic pig production will be described and submitted to the DARCOF III programme. If granted, this project will be managed by the group responsible for this project and will allow Annette Nygaard Jensen to continue her work related to food safety in organic pig production as a post doc.

C.2 Fulfilment of deliverables and milestones

(To be completed for each work package)

WP1: Investigation of introduction and spread of *Salmonella* in outdoor pigs	Time schedule according to application	Deviations, if any*	Full filled
Deliverables			
1. Determination of detection limit for bacteriological methods	Jan. 03		Yes
2. Information on the risk for *Salmonella* transmission to uninfected piglets constituted by infected animals and contaminated pasture environment representing different infection pressures	Sept. 03		Yes
3. Information on whether survival of *Salmonella* in the environment under naturally conditions is high enough to cause/transmit infection in uninfected animals	Sept. 03		Yes
4. Information on the pathogen reducing effect of ploughing pastures will be available if the infection level established is sufficiently high for this evaluation	Nov. 03	Evaluation not possible.	
5. Scientific and international publication of results	June 04	Overall presentation of salmonella data is in preparation, March 2005.	Partly
6. Practical guidelines for minimising the risk of salmonella infection in outdoor pig production	June 04	See D	
Milestones			
Determination of detection limit for the bacteriological method applied	After 1. quarter of 03		Yes

First experimental period – spread of infection between experimentally infected animals and uninfected tracer animals	After 2. quarter of 03		Yes
Second experimental period – spread of infection from pasture environment to uninfected tracer animals	After 3. quarter of 03		Yes
Third experimental period – *either* spread of infection from pasture environment to uninfected tracer animals and effect of soil treatment *or* spread of infection between experimental highly infected animals and uninfected tracer animals	After 3. quarter of 03		Yes
Scientific and international publication of results and formulation of practical guidelines for minimizing the risk of salmonella infection in outdoors pig production	After 2. quarter of 04	Publications in preparation, see D. March 2005	Partly

WP2: Investigation of the dynamics of natural *Campylobacter* infection in outdoor pigs	Time schedule according to application	Deviations, if any*	
Deliverables			
7. Development and evaluation of a new approach for determining the species composition (C. jejuni and C. coli) in faecal samples and subsequent isolation of the species in minority	Jan. 03		Yes
8. Publication of method in the form of a short paper (note/letter)	Apr. 03	Submitted 2004	Yes
9. Elucidation of the infection dynamics of thermophilic campylobacters in out-door organic pigs, including time of colonisation, excretion level in faeces, number of different strains co-colonising one piglet during time, persistence of strains, and the ratio between C. jejuni and C. coli in individual animals	Oct. 03		Yes
10. Information on the interaction between individual pigs and between the pigs and environment with respect to Campylobacter infection	Nov. 03	The serotype data needed to differentiate between species is incomplete, Febr. 2005	partly
11. Scientific and international publication of results	June 04	Delayed, see D, June 2005	No
Milestones			
Implementation of a PCR-based method for determination of the composition of *Campylobacter* species in faecal samples and the subsequent isolation of the species in minority. Determination of detection limit of the method.	After 2. quarter of 03		Yes
Results are obtained on the infection dynamics of thermophilic campylobacters in outdoor piglets repeated during three seasons	After 1. quarter of 04		Yes
Results are obtained on the interaction between outdoor piglets and the environment with respect to *Campylobacter* infections	After 1. quarter of 04	Serotyping incomplete, Febr. 2005	Partly

Publication of results in international journal	After 2. quarter of 04	Delayed, June 2005	No

** Deviations are to be further discussed in D*

D. Description of deviations and subsequent adjustments of plans

Ph.d.
A Ph.D. studium was established based on the project as explained in F. This has caused adjustment of the budget (see 8, A & B). Additionally, some of the planned activities have been delayed due to the time allocated for participation in Ph.D.courses, but will be finished after the project period during the Ph.D. Furthermore, related activities will be included in the study as a part of the Ph.D.

WP1.4 - *Effect of ploughing*. Evaluation of the pathogen-reducing effect of ploughing was not possible due to a large difference in the contamination level in "identical" pastures. In one pasture the contamination level was still high at the end of the second period, and the infectivity was evaluated by introduction of new non-infected tracer animals. In the other 3 pastures, the infection study from the first period was repeated, but with high inoculum dose only.

WP1.5. - *Publication of the salmonella infection study*. A part of the results, involving the introduction of salmonella and the small-scale wildlife study, has been accepted for publication. The overall presentation of the salmonella infection study is in preparation and will be submitted as a scientific paper as a part of the Ph.D. study.

WP1.6. - *Practical guidelines*. It was expected that examination of the 'ploughing effect' on salmonella-contaminated pastures would provide the information for such guidelines, however, these examinations were not practically feasible due to the big differences in the contamination level. Nevertheless, the results indicated the importance of eliminating pigs with signs of salmonellosis to limit the persistence of infections and good hygiene measures e.g. in the hut to reduce survival in the environment. This has been communicated in FØJOenyt, DARCOFenews and Økologisk Jordbrug but no specific guidelines have been formulated.

The incomplete fulfilment of the last milestone in WP1 is related to the deviations of the deliverable 1.5 and 1.6 described above.

WP2.8. - *Publication of method*. This deliverable was postponed because we chose to present the evaluation of the rt-PCR and the hybridization methods including the results of their applicability for detection of mixed campylobacter infections in pigs in one paper. Inclusion of the results for the naturally infected samples was assumed to further enhance the interest of the paper.

WP2.10. - *Interaction with the environment*. Examination of the possible interaction of pigs and environment with respect to Campylobacter infections requires further differentiation of the campylobacter species by serotyping. This work is labour intensive and has been delayed, but will be finished, as a part of the Ph.D. The inclusion of the small-scale wildlife study will provide additional information on potential interactions.

WP2.11. *Publication of the Campylobacter infection study*. The laboratory analysis of the obtained Campylobacter isolates is delayed as described above (WP2.10). However, preliminary results has been presented at the SAFE PORK Symposium, and the final results will be submitted as a scientific as part of the Ph.D study.
The incomplete fulfilment of the last two milestones in WP2 is related to the deviations of the deliverable 2.10 and 2.11 described above

Project publications and other products

1. Products from Organic Eprints archive

Peer-reviewed and accepted

English

Jensen, A.N.; Andersen, M.T.; Dalsgaard, A.; Baggesen, D.L. and Nielsen, E.M. (2005) Development of real-time PCR and hybridization methods for detection and identification of thermophilic Campylobacter spp. in pig faecal samples. *Journal of Applied Microbiology*.
Jensen, A.N.; Lodal, J. and Baggesen, D.L. (2004) High diversity of salmonella serotypes found in an experiment with outdoor pigs. *Wageningen Journal of Life Sciences* 52(2):pp. 109-117.

Not peer-reviewed

English

Jensen, Annette Nygaard and Baggesen, Dorte Lau (2004) Spread of salmonella in organic pigs. *Newsletter from Danish Research Centre for Organic Farming*(4). Online at <http://www.darcof.dk/enews/dec04/salmonella.html>
Jensen, Annette Nygaard and Nielsen, Eva Møller (2003) Campylobacter species distribution in outdoor pigs: Oral presentation O44. Paper presented at SAFEPORK 5th International Symposium on the Epidemiology and Control of Foodborne Pathogens in Pork, Hersonissos, Crete, Greece, October 1-4, 2003; Published in Leontides, Leonidas, Eds. *Proceedings. SAFEPORK 5th International Symposium on the Epidemiology and Control of Foodborne Pathogens in Pork.*, page pp. 134-136.

Dansk - Danish

Jensen, A.N. (2004) Fandt mange forskellige Salmonellatyper i forsøg med økogrise [Different salmonella types found in organic pig experiment]. In *Økologisk Jordbrug*, 24. december. December, No 327, page 6.
Jensen, A.N. and Baggesen, D.L. (2004) Salmonella bakterier kan spredes blandt økologiske grise [Salmonella can spread between organic outdoor pigs]. *FØJOenyt*(6). Online at <http://www.foejo.dk/enyt2/enyt/dec04/salmonella.html>
Jensen, A.N. and Baggesen, D.L. (2004) Salmonella og Campylobacter i økologisk svineproduktion [Salmonella and Campylobacter in organic pig production]. FØJO II Brochure .
Jensen, Annette Nygaard (2004) Risiko for salmonella ved økologisk griseproduktion [Risk of salmonella in organic pig production]. Klumme i Økologisk Jordbrug .

2. Other products (oral presentations, public meetings, field days, etc.)

Oral presentation. Bacterial infection risk associated with outdoor organic pig production - including a small survey of wildlife as a reservoir of *Salmonella* and *Campylobacter*. Seminar, Rodent Control Strategies in Organic Pig and Poultry Production Systems, 26-28 May, 2004, Wageningen, The Netherlands.

Participation. Enhancing animal health security and food safety in organic livestock production systems, 16th-18th Sept. 2004, SAFO (Sustaining Animal Health and Food Safety in Organic Farming) 3rd Workshop Falenty, Polen.

Planned:

Publications

Jensen, A. N., Dalsgaard, A., Nielsen, E. M. and D.L. Baggesen. Transmission and survival of *Salmonella* Typhimurium in an outdoor experimental set-up with organic pigs

Jensen, A. N., Dalsgaard, A., D.L. Baggesen and Nielsen, E.M. Campylobacter infection dynamics in outdoor organic pigs.

Poster presentation.
Enhancing animal health security and food safety in organic livestock production systems, 17th-19th March. 2005, SAFO (Sustaining Animal Health and Food Safety in Organic Farming) 4th Workshop , Switzerland.

Transmission and survival of *Salmonella* Typhimurium in an experiment with organic outdoor pigs. International Scientific Conference Organic Agriculture, ISOFAR, 21-23 September 2005, Adelaide, Australia

Campylobacter infection dynamics in organic pigs. The 13th International Workshop on Campylobacter, Helicobacter and Related Organism, CHRO 4-8 Sept. 2005, Gold Coast, Australia.

* 25-75% financed by DARCOF
** 5-25% financed by DARCOF

Scientific education

A Ph.D. study has been established based on the project. This has been possible due to an additional grant given by Research School for Organic Agriculture and Food Systems, The Royal Veterinary and Agricultural University and Danish Research Centre for Organic Farming.

The Ph.D. study lasts three years from 1st of October 2002 to 31st of September 2005. In addition to the activities described in this project the Ph.D. study will include a study of the seroprevalence of salmonella in Danish organic pig production based on data base information.

Cand. Scient. Annette Nygaard Jensen has been employed as a ph.d.-student. Associate professor Anders Dalsgaard, DVM, Ph.D., Dept. of Pathobiology, KVL, is main supervisor whereas Dorte Lau Baggesen, Senior research officer, Ph.D. and Eva Møller Nielsen, Ph.D. are co-supervisors.

National and international cooperation

The outdoor experiments were carried out at the research animal facilities of The Danish Veterinary and Agricultural University.
The examinations of rodents in the surrounding environment were carried out in cooperation with Jens Lodal, Danish Pest Infestation Laboratory.

Critical reflection on the project

The amount and quality of expected achievements will increase because of the establishment of a Ph.D. study with additional activities. The contact to other researchers in the area of organic farming will be improved through the participation in the Research School for Organic Agriculture and Food Systems.

The pigs used in the experiment were delivered by an organic farmer. It was believed that the organic feed and the outdoor life would influence the health condition and intestinal microflora of organic bred animals. Thus, for the most realistic picture of the infection dynamics in organic pigs, conventionally pigs should probably be avoided. Only rather few farmers had the capacity to deliver 56 pigs of similar age at one time, but a farmer was recommended by an agricultural consultant. The information about he herd was limited, besides a sampling of faecal samples from the herd to examine the salmonella status before the experiment. The pigs were delivered in connection to weaning at the age of 7 weeks, but there was a big variation in their weight, which may affect their resistance to infection, however, the results reflected apparently no clear correlation between weight and infection.
In the second period, cases of PMWS were experienced among the pigs and some pigs of poor condition were killed. This may of course also influence the infection dynamics of salmonella, as the pigs may be more prone to infection, but such potential interaction could not be controlled. Although it may seem like a "worst case scenario" it may reflect a rather realistic situation among organic pig farmers.

The post-mort examinations of all pigs were omitted because they were not practically feasible. Such examinations would have indicated the location of infection in the pig and perhaps allowed detection of carrier animals with intermittent excretion of salmonella. However, the bacteriological level of excretion in faeces is probably the most significant factor for the transmission of salmonella to non-infected animals.

The *Salmonella* inoculum strain was frequently detected in the environment of the pasture. Since the frequency of salmonella-positive samples was quite high, application of the semi-quantitative method for detection of salmonella would probably have enabled a better differentiation of the actual contamination load in each pasture. However, this method was labour-intensive and therefore not included.
In order to examine the non-host survival of salmonella over time, the bacteriological examination of soil and water samples continued after removal of the last pigs. After 7 weeks salmonella was detected rarely and we ended the examinations due to the high resource requirements. However, these results indicated that salmonella can persist in the environment and that good hygiene is necessary to eliminate salmonella. Cleaning of the hut including spread of lime is probably very important to reduce the persistence of salmonella. Since huts should be removed frequently already to minimize the Nitrogen load, such salmonella preventive cleaning probably has rather few implications for the organic farmer. Survival of salmonella in soil environments for over a year has been reported. It is influenced by numbers of physical and chemical factors and may be prolonged at low temperatures. Whether a salmonella-contaminated environment at certain favourable conditions can constitute a 'new' infection risk long after removal of salmonella-infected animals is not sure. It was considered to introduce new tracer-animals in the spring 2004, but due to the mainly low infection rate in the second and third experimental period (2003), it was assumed that the risk of infection would be very low. Moreover, we would still be unable to exclude that some favourable but to us unknown circumstances could cause infections in introduced pigs.

The unexpected detection of different salmonella serotypes in addition to the inoculum strain, promoted the inclusion of a small-scale wildlife survey in collaboration with the Pest Infestation Laboratory in the search of the potential source of these serotypes. However, no salmonella was detected in approximate 40 rodents and crow-birds leaving the source of these serotypes an open question yet to be elucidated. A previous study on salmonella in wildlife also found little salmonella. Nevertheless, detection of the different types confirms that salmonella bacteria is widespread in the environment. Inclusion of this study provided the opportunity to present the results

at a Dutch seminar on 'Rodent control strategies in organic farming'.
Detection of campylobacter in crow-birds and rats will add information about the potential interactions between campylobacter in pigs and the environment.

8. Budget

A. Account for any change in budgets

B. Budget for the whole project (1.000 DKK)

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Man-months					
Scientific personnel	24	3	12	9	24
Technical personnel	19		19		19

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Salaries	1135	80	750	301	1131
Scientific personnel	600	80	370	301	751
Technical personnel	535		380		380
Operational costs	532	35	462	38	535
Direct costs	1667	115	1212	339	1666
Indirect costs (20% of direct costs)	333	23	242	67.8	333
Total	2000	138	1454	406.8	1999

Comments:

The total salary consumption for the scientific (VIP) and technical (TAP) personnel equals the original budget approximately, but the actual expenses have been higher and lower for VIP and TAP, respectively, than expected. This explains the discrepancy between the original budget and total consumption for each salary group.

9. Signatures and stamps

Name	Institute	Date	Signature
Head of project Dorte Lau Baggesen	Danish Institute of Food and Veterinary Research		

Appendix I. Detailed budget

A. Budget for each participating institute (1.000 DKr)

Name of Institute:

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Man-months Scientific personnel Technical personnel					

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Salaries Scientific personnel Technical personnel					
Other operational costs					
Equipment					
Others (please specify)					
Direct costs					
Indirect costs (20% of direct costs)					
Total					

Comments:

B. Budget for each participating department (1.000 DKK)

Name of Institute and department:

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Man-months Scientific personnel Technical personnel					

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Salaries Scientific personnel Technical personnel					
Other operational costs					
Equipment					
Others (please specify)					
Direct costs					
Indirect costs (20% of direct costs)					
Total					

Comments:

C. Budget for co-financing from each participating institute (1.000 DKK)

Name of Institute:

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Man-months Scientific personnel Technical personnel					

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Salaries Scientific personnel Technical personnel					
Other operational costs					
Equipment					
Others (please specify)					
Direct costs					
Indirect costs (20% of direct costs)					
Total					

Comments: