

Title: Bacterial infection risk associated with outdoor organic pig production with special reference to *Salmonella* and *Campylobacter* infection

FØJO II project no. 38

Acronym: SaCaFree

Date: 25 November 2001

Summary in Danish:

Den moderne forbruger er i stigende grad kritisk overfor kvaliteten af fødevarer af animalsk oprindelse. Den øgede fokus på husdyrproduktionen og dyrevelfærd har betydet en øget interesse for økologisk produktion, udendørs husdyrbrug og andre alternative produktionssystemer, hvor der er lav dyreintensitet og gode muligheder for dyrene til at udtrykke naturlig adfærd. Generelt forventer forbrugeren også, at disse produkter har en højere mikrobiologisk kvalitet sammenlignet med produkter fra konventionelle produktionssystemer. Der er dog i dag ingen dokumentation for at økologiske produkter har et lavere indhold af de almindelige zoonotiske bakterier (f.eks. *Salmonella*, *Campylobacter* og *Yersinia*).

Formålet med det ansøgte projekt er at forbedre vores viden om risikoen ved udendørs svineproduktion i relation til spredning og persistens af *Campylobacter* og *Salmonella* infektioner. De specifikke mål vedrørende *Salmonella* er, at undersøge overlevelse af *Salmonella* Typhimurium i jord og græs fra kontaminerede folde brugt til udendørs svineproduktion, måling af infektiviteten af naturligt kontaminerede folde over tid, og – i tilfælde af høj infektivitet – evaluering af den patogenreducerende effekt af jordbehandling. For termofile *Campylobacter* er formålet, at beskrive infektionsdynamikken over tid for naturligt forekommende *Campylobacter* infektioner i udendørs svin, inklusiv kolonisationstidspunkt, udskillesniveau, artsfordeling i gruppen og i individer, interaktion med miljøet, samt den mulige ændring af forekomst og artsfordeling over tid og i relation til kontaminerede miljøer.

Undersøgelserne vil blive gennemført i forsøgsfolde med økologisk fodrede smågrise. Eksperimentelt salmonellainficerede smågrise vil blive blandet med ikke inficerede smågrise med henblik på at følge spredningen af infektionen. I forbindelse hermed vil jordprøver blive undersøgt mht. fastlæggelse af kontaminationsniveauet. Efter to måneder bliver grisene slagtet og nye grise indsat på foldene. Dyr såvel som miljøprøver vil blive undersøgt for forekomst af *S. Typhimurium* ved kvantitative og kvalitative bakteriologiske metoder ligesom dyrenes vil blive fulgt gennem serologisk undersøgelse for *Salmonella*. På grundlag af resultater opnået i den anden forsøgsperiode, vil der i tredje periode blive gennemført yderligere undersøgelser til belysning af smittespredningen. Disse vil, hvis der er opbygget et højt smitteniveau, omfatte undersøgelser af den smittereducerende effekt af pløjning.

En metode vil blive udviklet med henblik på at fastlægge den relative fordeling af *C. jejuni* og *C. coli* i fæcesprøver og efterfølgende isolation af den sjældnest forekommende species. De grupper af grise, der fungerer som kontrol for salmonella undersøgelserne, vil indgå i studier af infektionsdynamikken for naturligt forekommende campylobacterinfektioner hos udendørs grise. Grisene vil løbende blive undersøgt for naturlig kolonisation af *Campylobacter* herunder bl.a. fastlæggelse af den relative forekomst af *C. jejuni* og *C. coli*. I tilknytning hertil vil også miljøprøver blive undersøgt for forekomst af termofile campylobacter. Isolater fra grise og miljø vil blive species identifi-

ceret og serotypen fastlagt, hvorved infektionsdynamikken beskrives (antal forskellige stammer i de enkelte grise, persisterende smitte eller skift af typer gennem observationsperioden)

De opnåede resultater vil danne udgangspunkt for formulering af retningslinier for økologisk svineproduktion med henblik på at begrænse risikoen for zoonotiske infektioner. Resultaterne vil endvidere blive publiceret i internationale tidsskrifter. En stor del af de opnåede resultater vil være af kvantitativ karakter, hvilket der pt. er stort behov for i forbindelse med udarbejdelse af risikoanalyse for zoonotiske infektioner i økologisk svineproduktion.

1. 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 the proposed project is 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 are 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, and in the case of high infectivity, evaluation of the pathogen reducing effect of soil treatment. For thermophilic *Campylobacter*, the objectives are 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 will be set up. Experimentally infected piglets will be grouped together with uninfected tracer piglets in order to monitor the transmission of infection. In addition, soil samples will be analysed to determine the level of contamination. After a period of two months, the pastures will be vacated, infected piglets slaughtered and tested. A new set of uninfected piglets will be introduced in each pasture to monitor if these animals become infected due to their habitation in the *Salmonella* contaminated pastures. The pastures and animals will be sampled and monitored (bacteriological (qualitatively and semi-quantitatively), serological and by necropsy examinations). Based on the results obtained in the second period, a third period of two months will be initiated for further monitoring of the infectivity of the *Salmonella* contaminated pasture environment, including the preventive effect of ploughing the pastures before new tracer animals are introduced.

A method will be 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 serving as controls in the experimental *Salmonella* infection study will also be used for studying the dynamics of natural *Campylobacter* infection in outdoor pigs. The piglets will be monitored for natural colonisation of thermophilic *Campylobacter* species, and the ratio between *C. jejuni* and *C. coli*. Likewise, environmental samples are analysed throughout the experimental periods. Isolates from animals and environment will be 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 achievements obtained will be formulated in practical guidelines directly applicable for the organic pig producers in order to minimise 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.

2. Research group

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Scientific assistant (NN) not employed yet

3. Introduction

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 lead 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 such as locomotion, foraging, exploration and nest building. From a health point of view such production systems are characterised by e.g. less problems with respiratory diseases compared to intensive in-door production.

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. It is expected that meat from alternative production systems in addition to be free of drug residues and pesticides also is free from zoonotic pathogens and therefore constitute a lower risk to human health.

4. State of the art

Today, there is no available documentation for a lower level of zoonotic infections, e.g. salmonella infection, in organic or other alternative animal productions systems. Only few prevalence studies of zoonotic infections such as salmonella and campylobacter infections have included herds of alternative productions systems. Mainly due to the small number of these herds, low strength of the results is obtained from such studies.

In a study of the salmonella-seroprevalences in Danish organic, free-range, conventional and breeding pig herds, Wingstrand *et al.* (1999) showed that there was an OR of 1.7 for meat juice samples from both organic and free-range herds being seropositive compared to samples from conventional herds. The result was significant for the free-range herds ($p=0001$) but due to limited number of samples not significant for organic herds. Similar results were obtained in a Dutch study where the salmonella seroprevalence in free-range finishers was significantly higher (44,6%) than in inten-

sively housed finishers (24,5%) (Wolf *et al.*, 2001). The prevalence was also higher in biologic-dynamic finishers pigs (37,5%) but again this result were not significant due to the limited numbers of samples.

Campylobacter is widely prevalent in conventional pig herds (Weijtens *et al.* 1993). Preliminary results from Switzerland indicate that this is also the case in herds with animal friendly production systems (Regula *et al.*, 2001). There is, therefore, a general need for information on the impact of such new alternative production systems on the prevalence of zoonotic pathogens as *Salmonella* and *Campylobacter*.

Many different factors influence the establishment of salmonella or campylobacter infection in a pig herd. Compared to conventional production some specific management procedures of the alternative productions systems influence the occurrence of these infections. In general, the animals in the alternative systems should benefit from a lower animal density and possibly a higher general resistance to infections.

The major source of introduction of *Salmonella* into a pig herd is subclinically infected animals. In conventional production, feeding have proved to be important for the establishment of infection as non-heat-treated meal lowers the prevalence of *Salmonella* in finishers compared with pelleted feed (Jørgensen *et al.*, 1999). Also the level of protein may influence the establishment of *Salmonella* infection (Pedersen *et al.*, 2000) and the high protein level in organic feed could be a risk in connection to infection in organic pig production. The possible interactions between feed and salmonella infection in finishers raised on organic feed will be evaluated in another project carried out by the present group ("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).

An important difference between the different kinds of alternative pig production systems and conventional production is the requirement of access to outdoor areas, partly or in the entire production period. This means that the pigs have contact with the external environment, which can constitute a risk of infection.

Several investigations have demonstrated persistence of salmonella infection in pig farms for several months and even years (Baggesen *et al.*, 2000; Sandvang *et al.*, 2000; Davies & McLaren, 2001). Even though it is difficult to differentiate between persistence caused by subclinically infected animals and persistence caused by continuous contamination of the environment, isolations of *Salmonella* from soil, slurry, manure and equipment indicated that contaminated environment constituted a risk for infection.

A possible source of infection for outdoor pigs can also be contact to wildlife or other kinds of non-production animals, which can be infected with zoonotic bacteria as *Salmonella* and *Campylobacter*. The non-porcine animals may constitute a reservoir of infection from where the bacteria are spread directly or indirectly to the pigs. Preliminary results from an ongoing Danish project (Wildlife as a source of salmonella infection in food-animal production; FØSI00-SVS-6) carried out by the present group indicate that non-production animals seems to play a minor role in relation to spread of salmonella-infection to conventional pig herds. In contrast, the preliminary data from this project shows a high prevalence of *Campylobacter* in non-production animals, and the unfinished subtyping data indicate transmission of *Campylobacter* between production animals and non-production animals. Possibly, the direction of this transmission can be two-way. For free-range pigs the transmission between pigs and wild-living animals are likely to be of more importance.

Among human clinical cases of campylobacteriosis in developed countries, *C. jejuni* is the dominant species (in Denmark 94%) and *C. coli* is less common (in Denmark 6%) (Nielsen *et al.*, 1997).

This dominance of *C. jejuni* is also found in most healthy carrier animals, e.g. broiler chickens, cattle, and wild-living birds and mammals (Nielsen *et al.* 1997; Petersen *et al.* 2001). In contrast, most studies in pigs have shown a strong dominance of *C. coli* (Manser and Dalziel 1985; Munroe *et al.* 1983), and each year the Danish national surveillance programme show more than 95% *C. coli* among thermophilic campylobacters isolated from caecal samples of conventional pigs at slaughter (Annual Report, 2001). However, some American studies show that pigs can harbour *C. jejuni* as frequently or more frequent than *C. coli*. In one specific hog farm, the prevalence of thermophilic campylobacters was 76-100% for gilts and pregnant sows, and 76-87% of these were *C. jejuni* (Young *et al.* 2000). In a study of four farms, thermophilic campylobacters were isolated from 70-100% of the pigs, and *C. jejuni* constituted an average of 31% of these (ranged from 0 to 76% depending on the farm) (Harvey *et al.*, 1999). No data is available for the species distribution of campylobacters in free-range pigs. However, it can be speculated that free-range pigs in Denmark harbour more *C. jejuni* as they are in closer contact with the environment and wild-living animals where the ratio of *C. jejuni* to *C. coli* is higher. *C. jejuni* is considered more virulent to humans and therefore a shift towards more *C. jejuni* in the pig production is of potential importance to food safety.

Individual pigs are often colonised with several *Campylobacter* strains (e.g. several different serotypes or genotypes can be isolated from the same animal) and sometimes both *C. coli* and *C. jejuni* are found in the same sample by conventional culture techniques (Madden *et al.* 2000; Weijtens *et al.* 1999). However, it is likely that pigs are often colonised with *C. jejuni* as a minority community compared to a much more abundant *C. coli* community. This will usually not be found by conventional culture procedures where 1-5 colonies are identified. However, it has been shown that a sub-optimal culture medium for growth of *C. coli* facilitates the growth and detection of other species from the porcine intestinal tract (Madden *et al.*, 2000). Thus, it was found that the species-distribution changed significantly depending on the technique and the media used. The presence of *C. jejuni* in the intestinal tract – although they are outnumbered by *C. coli* – could be of importance for the food safety as *C. jejuni* generally have a better survival rate in extra-intestinal environments (Thomas *et al.* 1999). For example, an increasing relative occurrence of *C. jejuni* compared to *C. coli* was found during the slaughter process in three Danish slaughterhouses: from 0.3% *C. jejuni* (the remaining being *C. coli*) in faeces to 11% *C. jejuni* on the swine carcasses after chilling (Sørensen and Christensen, 1996).

Campylobacter is usually considered a natural part of the porcine intestinal microbiota. It has been shown that conventionally raised piglets become colonised with campylobacters during the first few hours and weeks after birth (Weijtens *et al.* 1997; Young *et al.* 2000). The concentration of campylobacters increases from birth to a maximum at weaning (on average 10^7 cfu/g) and then stabilises at a lower level in adult pigs (10^5 cfu/g) (Young *et al.* 2000). The dynamics of campylobacter colonisation in alternative production systems have not been investigated. It is possible that there are significant differences in the prevalence as well as the number of campylobacters colonising the intestinal tract of free-range pigs, e.g. these might have a more diverse microbiota that can reduce the level of campylobacters. The persistence and exchange of *Campylobacter* strains might also be different due to the presumable lower infection pressure from the other pigs, but higher infection pressure from other sources.

Salmonella can persist in extra-animal environment but the level will decline depending on UV-light, heat and drying. The dissemination time (T_{90}) of *Salmonella* Typhimurium on grass has been shown to vary between 24 days nearest the ground (0-8 cm) and 18 days in the top (>16 cm) as a result of the UV light (Schlundt, 1982). Whether a contamination of *Salmonella* in the environment, e.g. on the pasture, will constitute a specific risk and result in an infection of outdoor pigs will therefore depend on the actual contamination level, the time and environmental reduction and the sensitivity of the pigs.

In Denmark, multiresistant-trimetoprim resistant *Salmonella* Typhimurium DT104 has been spread to outdoor pig herds with no known contact but located less than one kilometre from each other (Baggesen, unpublished results, 2001). It has not been possible to identify the specific route of infection, but the environment of the single herd was contaminated for several months and the spread may have been transmitted by wildlife moving from one pasture to another.

5. 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 outdoor 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 infection 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 environment.

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).

6. Description of workpackages including methods

Table 1: Workpackage list

WP No	WP title	Responsible participant	Budget in 1000 DKr*	Start	End	Deliverable, No
1	Investigation of introduction and spread of <i>Salmonella</i> in outdoor pigs	Dorte Lau Baggesen	1,324.8	July 2002	June 2004	1-6
2	Investigation of the dynamics of natural <i>Campylobacter</i> infection in outdoor pigs	Eva Møller Nielsen	675.6	July 2002	June 2004	7-12
Total			2,000.4			

* All expenses concerning the animal experiments (756,000 DKr.) are covered by WPI. These experiments form the basis for both WP's.

Table 2: Description of workpackages

WP1: Investigation of introduction and spread of *Salmonella* in outdoor pigs

Workpackage number:	1
Start date or starting event:	July 2002
Responsible person:	Dorte Lau Baggesen
Contributing persons:	Eva Møller Nielsen, NN
Person-months:	24

Objectives:

- To evaluate the survival of *Salmonella* Typhimurium in soil and grass of contaminated weaning and fattening pastures for outdoor pig production systems
- To measure the infectivity of naturally contaminated pastures in relation to time
- In the case of high infectivity, to evaluate the pathogen reducing effect of soil treatment

Description of work:

1. Laboratory studies will be carried out in order to determine the detection limit of the culture methods.
2. Setting up of an experimental organic pig unit based on current EU guidelines as well as existing common outdoor organic herd management practices. Setting up of 6 outdoor pastures with the following *Salmonella* infection strategy:

Pasture 1 & 2: 5 uninfected piglets inoculated with 10^7 cells of a specific *Salmonella* Typhimurium strain placed together with a group of 5 uninfected piglets without *Salmonella* inoculation.

Pasture 3 & 4: 2 piglets with 10^7 *Salmonella* and 8 without *Salmonella* in each pasture.

Pasture 5 & 6: All 10 piglets with no *Salmonella* (control).

All piglets will be ca. 25-30 kg each.

3. First period: The piglets will be monitored for a 2-month cycle by taking bacteriological, serological and necropsy samples. In addition to conventional qualitative methods, the bacteriological methods will include quantitative or semi-quantitative methods for evaluation of the magnitude of excretion from infected animals and contamination in the environment. The employed serological methods are identical to the methods applied in the national surveillance, which have

proven to be sensitive against *S. Typhimurium* antibodies.

4. Second period: After the first period of 2 months, pastures will be vacated, infected piglets slaughtered and tested. A new set of 10 uninfected piglets will then be introduced in each pasture to monitor if these uninfected animals get infected due to their habitation in the *Salmonella* contaminated pastures, for another period of 2 months. The pastures and animals will be sampled and monitored in a similar way (bacteriological (qualitatively and semi quantitatively), serological and necropsy examinations).
5. Third period: Based on the results obtained in period 2 (if *Salmonella* infects the animals) a third period of 2 months will be initiated for further monitoring the infectivity of the *Salmonella* contaminated pasture environment as described in d. The spread of *Salmonella* infection in the uninfected tracer piglets will provide a picture of the infectious potential of *Salmonella* through contaminated environment, time lapse and other relevant parameters (including the presence of “hot spots” of pasture contamination/pathogen survival etc.). This third period will, however, only include 3 pastures one from each of the groups A, B and C.

Third period: Parallel to 5., the soil in the three remaining pastures (one from each of the groups A, B, and C) will be treated by plough before uninfected trace animals will be introduced as described in 4.

Alternatively in case *Salmonella* do not infect the animals in period 2, a third period with introduction of more experimentally highly infected pigs among uninfected tracer pigs will be carried out.

6. Evaluation of data, formulation of practical guidelines and publication of results

Deliverables:

New scientific knowledge will be gained, which subsequently can be used directly for reduction of the spread of *Salmonella* in outdoor pig production systems:

1. Determination of detection limit for bacteriological methods
2. Information on the risk for *Salmonella* transmission to uninfected piglets constituted by infected animals and contaminated pasture environment representing different infection pressures.
3. Information on whether survival of *Salmonella* in the environment under naturally conditions is high enough to cause/transmit infection in uninfected animals
4. Information on the pathogen reducing effect of ploughing pastures will be available if the infection level established is sufficiently high for this evaluation
5. Scientific and international publication of results
6. Practical guidelines for minimising the risk of salmonella infection in outdoor pig production

Milestones:

1. Determination of detection limit for the bacteriological method applied
2. First experimental period – spread of infection between experimentally infected animals and uninfected tracer animals
3. Second experimental period – spread of infection from pasture environment to uninfected tracer animals
4. 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
5. Scientific and international publication of results and formulation of practical guidelines for

minimizing the risk of salmonella infection in outdoors pig production

WP2: Investigation of the dynamics of natural *Campylobacter* infection in outdoor pigs

Workpackage number: **2**
 Start date or starting event: **July 2002**
 Responsible person: **Eva Møller Nielsen**
 Contributing persons: **Dorte Lau Baggesen, NN**
 Person-months: **17**

Objectives:

- To describe the prevalence and excretion level of natural campylobacter infection over time in outdoor pigs
- To describe the species and serotype distribution in the group and in the individuals
- To evaluate the possible changes in prevalence and species distribution in relation to time and environmental contamination
- To describe the interaction between infected animals and contaminated environment

Description of work:

Groups of pigs serving as controls in WP1 (pastures 5 and 6) will be included in WP2.

7. Implementation of methods for detection of *C. jejuni* in faecal samples from pigs where *C. coli* outnumber *C. jejuni*. The detection method will be based on a species specific PCR-assay (TaqMan PCR) that is already developed in our laboratory for identification purposes. A similar TaqMan PCR assay is implemented in the laboratory for detection of other organisms in faecal samples. Ten-fold dilution of faecal samples will be examined by PCR for both *C. jejuni* and *C. coli*. Subsequent isolation of *C. jejuni* will be done for verification by two approaches: 1) When the PCR assay indicates 10 fold or less *C. jejuni* compared to *C. coli*: isolation by colony hybridisation with *C. jejuni* specific probes; 2) When *C. jejuni* is more abundant or at the same level as *C. coli*: up to 5 colonies from two different culture media will be identified.
8. Setting up of 2 outdoor pastures (Salmonella-negative control pastures described in WP1): Ten 8-week old piglets in each pasture are set out and kept for a time period of approx. 8 weeks during spring.
 The piglets will be monitored weekly for natural colonisation of thermophilic *Campylobacter* species. The first sampling will take place immediately before the piglets are set on pasture:
 - Detection, enumeration and isolation of thermophilic campylobacters by direct culture on selective medium (CCDA, 42°C, microaerophilic atmosphere) to establish the time of colonisation (if *Campylobacter* free when set out) and the number of *Campylobacter* excreted in faeces monitored over time
 - Monitoring of the ratio between *C. jejuni* and *C. coli* by PCR-detection of *C. jejuni* and *C. coli* in 10-fold dilutions of faecal samples
 - Specific isolation of *C. jejuni* in selected samples: either by colony hybridisation or by picking 5+5 colonies from an appropriate dilution using two selective media
 - Up to 10 isolates from culture positive animals will be identified to the species level and serotyped (heat-stable 'Penner' serotyping) to monitor the dynamics of the infection: number of different strains in each pig, persistence/exchange of strains over time.
9. Environmental samples will be taken weekly to monitor the level of campylobacters in the soil.

Samples from the water troughs will be cultured quantitatively for campylobacters. Semi-quantitative detection of low numbers of campylobacters in water and soil will be done by the use of enrichment cultures. Isolates from soil and water will be identified and serotyped as described for the faecal isolates. The species and type distribution in the environment and in the pigs will be compared. Strains that survive better in the environment will possibly be identified.

10. After the first period of 2 months, pastures will be vacated and piglets slaughtered as described in WP1. A new set of 10 uninfected piglets will then be introduced in each pasture as a repeat of first period in another season (summer) and to investigate the impact of contaminated soil on the infection of a new set of piglets.
11. A third repeat will take place in the autumn months.
12. Data analysis and writing of a manuscript for publication in a peer-reviewed international scientific journal.

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
8. Publication of method in the form of a short paper (note/letter)
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
10. Information on the interaction between individual pigs and between the pigs and environment with respect to *Campylobacter* infection
11. Scientific and international publication of results

Milestones:

5. 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.
6. Results are obtained on the infection dynamics of thermophilic campylobacters in outdoor piglets repeated during three seasons
7. Results are obtained on the interaction between out-door piglets and the environment with respect to *Campylobacter* infections
8. Publication of results in international journal

7. Implementation and time schedule

Table 3: Deliverables list

Deliverable, No	Deliverable title	Delivery date
1.	Determination of detection limit for bacteriological methods	Jan. 03
2.	Information on the risk for <i>Salmonella</i> transmission to uninfected piglets constituted by infected animals and contaminated pasture environment representing different infection pressures	Sept. 03
3.	Information on whether survival of <i>Salmonella</i> in the environ-	Sept. 03

	ment under naturally conditions is high enough to cause/transmit infection in uninfected animals	
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
5.	Scientific and international publication of results	June 04
6.	Practical guidelines for minimizing the risk of salmonella infection in outdoor pig production	June 04
7.	Development of a method for determining the <i>Campylobacter</i> species composition in faeces	Jan 03
8.	Publication of method	Apr 03
9.	Information on the infection dynamics of thermophilic campylobacters in out-door organic pigs	Oct. 03
10.	Information on the interaction between individual pigs and between the pigs and environment with respect to <i>Campylobacter</i> infection	Nov. 03
11	Scientific and international publication of results concerning <i>Campylobacter</i> infection dynamics	June 04

Table 4: Timetable

TI- TLE	Co-ordination	Quarter	2001*				2002*				2003*				2004*				2005*			
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	WP1:																					
1	Evaluation of laboratory methods – determination of detection limit																					
2	Establishment of experimental set up																					
3-5	3 periods of experimental infections																					
3-5	Laboratory investigations and data evaluation																					
6	Data evaluation and publication																					
M1	Determination of detection limit																					
M2-4	Monitoring spread of <i>Salmonella</i> in three experimental periods																					
M5	Practical guide lines and publication of results																					
	WP2:																					
7	Implementation of method																					
8, 10-11	Monitoring of <i>Campylobacter</i> in piglets in three periods																					
9	Monitoring of environmental samples																					
12	Data analysis and publication																					
M5	Implementation of method																					
M6-7	Results of investigation of infection dynamics and interactions																					
M8	Publication of results																					

* If convenient, indicate the actual month (can be done by numbers: January is 1 etc.)

8. Collaborative partners

The outdoor experiments will be carried out at the research animal facilities of The Danish Veterinary and Agricultural University. Expenses will be covered via the budget of the Danish Veterinary Institute as indicated below.

9. Budget (in 1000 DKr.)

Danish Veterinary Institute

Institution 1	2001	2002	2003	2004	Total
Months (scientific)		1 SRO*	2 SRO 12 RA*	1 SRO	
Months (technical)		3 LT*	12 LT 7 AA*	3 LT	
Salary (scientific)		45	90 420	45	180 420
Salary (technical)		60	240 175	60	360 175
Operation – animal experiments		10	287		297
Operation – laboratory		40	130	65	235
Overhead		31	268,4	34	333,4
Total		186	1610,4	204	2000,4

* Senior research officer (SRO), research assistant (RS), laboratory technician (LT), animal assistant (AA)

Institution 2	2001	2002	2003	2004	2005
Months (scientific)					
Months (technical)					
Salary (scientific)					
Salary (technical)					
Operation – equipment					
Operation - other					
Overhead					
Total					

10. References

1. Ann. (2001) Annual Report of Zoonoses in Denmark in 2000. Danish Zoonosis Centre, Danish Veterinary Laboratory, Copenhagen, Denmark.
2. Baggesen, D.L., Sandvang, D. and Aarestrup, F.M. (2000). Characterisation of *Salmonella enterica* serovar Typhimurium DT104 isolated from Denmark and their comparison with isolates from Europe and the USA. *Journal of Clinical Microbiology* 38 (4), 1581-1586.
3. Davies, R.H., McLaren, I.M. (2001). A six year study of the persistence of *Salmonella* Typhimurium DT104 on a Farrow to Finish pig farm. In: Proceedings of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and other food borne pathogens in Pork, p. 265-273. 2.-5. September, 2001, Leipzig, Germany.
4. Harvey, R.B., Young, C.R., Ziprin, R.L., Hume, M.E., Genovese, K.J., Anderson, R.C., Droleskey, R.E., Stanker, L.H., Nisbet, D.J. (1999). Prevalence of *Campylobacter* spp isolated from the intestinal tract of pigs raised in an integrated swine production system. *Journal of the American Veterinary Medical Association* 215, 1601-4.
5. Jørgensen, L., Dahl, J., Wingstrand, A. (1999). The effect of feeding pellets, meal and heat treatment on the salmonella-prevalence in finishing pigs. In: Proceedings of the 3rd International Symposium on the Epidemiology and Control of *Salmonella* in Pork, p. 308-312. August 5.-7., 1999, Washington DC, USA.
6. Madden, R.H., Moran, L. and Scates, P. (2000) Optimising recovery of *Campylobacter* spp. from the lower porcine gastrointestinal tract. *Journal of Microbiological Methods* 42, 115-119.
7. Manser, P.A. and Dalziel, R.W. (1985) A survey of campylobacter in animals. *Journal of Hygiene* 95, 15-21.
8. Munroe, D., Prescott, J.F. and Penner, J.L. (1983) *Campylobacter jejuni* and *Campylobacter coli* serotypes isolated from chickens, cattle, and pigs. *Journal of Clinical Microbiology* 18, 877-881.
9. Nielsen, E.M., Engberg, J. and Madsen, M. (1997) Distribution of serotypes of *Campylobacter jejuni* and *C. coli* from Danish patients, poultry, cattle and swine. *FEMS Immunology and Medical Microbiology* 19, 47-56.
10. Pedersen, A.Ø., Dahl, J., Borg, B. (2000) Effekt af Tylosin og foderets proteinindhold på forekomst af diarré hos slagtesvin [Effect of Tylosin and protein content in feed on the occurrence of diarrhoea in finishers], Information from "Landsudvalget for svin" no. 488, 14/9 2000.
11. Petersen, L., Nielsen, E.M., Engberg, J., On, S.L. and Dietz, H.H. (2001) Comparison of genotypes and serotypes of *Campylobacter jejuni* isolated from Danish wild mammals and birds and from broiler flocks and humans. *Applied and Environmental Microbiology* 67, 3115-3121.
12. Regula, G., Choizat, B., Danuser, J., Stärk, K.D.C., Ledergerber, U., Jemmi, Th (2001). Prevalence of *Salmonella* in fattening pigs and pork from animal friendly farms. In: Proceedings of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and other food borne pathogens in Pork, p. 217-219. 2.-5. September, 2001, Leipzig, Germany.
13. Sandvang, D., Jensen, L.B., Baggesen, D.L., Baloda, S.B. (2000). Persistence of a *Salmonella enterica* serotype Typhimurium clone in Danish pig production units and farmhouse environment studied by pulsed field gel electrophoresis (PFGE). *FEMS Microbiology Letters*, 187, 21-25.
14. Schlundt, J. (1982). Sygdomsfremkaldende tarmbakteriers overlevelse i biogasanlæg og på gyllebehandlede marker. Ph.D. thesis, The Royal Veterinary and Agricultural University.
15. Sørensen, R., Christensen, H. (1996). *Campylobacter* i svinekød - et problem? [*Campylobacter* in pork - a problem ?] *Alimenta* 19 (4), 9-11.
16. Thomas, C., Hill, D.J. and Mabey, M. (1999) Evaluation of the effect of temperature and nutrients on the survival of *Campylobacter* spp. in water microcosms. *J. Appl. Microbiol.* 86, 1024-1032.
17. Weijtens, M.J., Reinders, R.D., Urlings, H.A. and Van der, P.J. (1999) *Campylobacter* infections in fattening pigs; excretion pattern and genetic diversity. *J. Appl. Microbiol.* 86, 63-70.

18. Weijtens, M.J., Van der, P.J., Bijker, P.G., Urlings, H.A., Koster, D., van Logtestijn, J.G. and Huis, i., V (1997) The transmission of campylobacter in piggeries; an epidemiological study. *J. Appl. Microbiol.* 83, 693-698.
19. Weijtens, M.J.B.M., Bijker, P.G.H., van der Plas, J., Urlings, H.A.P. and Biesheuvel, M.H. (1993) Prevalence of campylobacter in pigs during fattening; an epidemiological study. *Veterinary Quarterly* 15, 138-143.
20. Wingstrand, A.; Dahl, J.; Lo Fo Wong, D.M.A. (1999) Salmonella-prevalences in Danish organic, free-range, conventional and breeding herds. In: Proceedings of the 3rd International Symposium on the Epidemiology and Control of *Salmonella* in Pork, p. 186-189. August 5.-7., 1999, Washington DC, USA.
21. van der Wolf, P.J., Elbers, A.R.W., van der Heijden, H.M.J.F., van Schie, F.W., Hunneman, W.A., Tielsen, M.J.M. (2001). *Salmonella* seroprevalences at the population and herd level in pigs in The Netherlands. *Veterinary Microbiology*, 80, 171-184.
22. Young, C.R., Harvey, R., Anderson, R., Nisbet, D. and Stanker, L.H. (2000) Enteric colonisation following natural exposure to *Campylobacter* in pigs. *Research in Veterinary Science* 68, 75-78.

Appendix: CV's of central persons, and description of role, qualifications, capacity and experience of each participant including maximum 5 relevant papers (max. 5 pages).

CV for Dr. Dorte Lau Baggesen

Born: 17. May 1962

Qualifications:

DVM, Ph.D.

Senior Scientist and Head of Section on *Salmonella* diagnostic and characterisation at the Danish Veterinary Laboratory (DVL). Dorte Lau Baggesen has since 1994 been responsible for the diagnostic work which in 2000 included microbiological examination of approximately 30.000 samples and epidemiological characterisation of approximately 18.000 *Salmonella* isolates by serotyping, phage typing and antibiogram typing. The results obtained are included in the Danish National Surveillance Systems, which cover monitoring of the spread of *Salmonella* from animal production through food to humans. In addition, molecular typing is performed in order to trace the spread of specific clones especially used in investigation of foodborne outbreak of salmonellosis.

As an integrated part of the National *Salmonella* Control Programme in Danish pig production, Dorte Lau Baggesen has participated in several research projects on epidemiology and control of *Salmonella* at herd level. An experimental model of subclinical *Salmonella* infection in pigs has been developed. This model has been used to evaluation the influence of vaccines and antibiotic growth promoters on *Salmonella* infection. The model has also been employed in studies on the interaction between helminths and *Salmonella*.

Expertise:

DVL is the national reference of *Salmonella* (NRL) in relation to the Directive 92/117/EEC.

Description of role and capacity to provide contribution to the project

Dorte Lau Baggesen will be the project leader of the applied project and responsible for the animal experiments and laboratory procedures concerning *Salmonella* (WP1).

The project has access to a well-equipped laboratory for microbiological investigations and epidemiological characterisation of *Salmonella* isolates, including definitive methods as sero- and phagotyping and molecular methods as plasmid analysis, PFGE and AFLP.

Dorte Lau Baggesen is project leader for two related projects: Wildlife as a source of salmonella infection in food-animal production, FØSI00-SVS-6, and "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

List of five recent publications relevant to the project.

Baggesen, D.L., Wegener, H.C., Bager, F., Stege, H., and Christensen, J. Herd prevalence of *Salmonella enterica* infections in Danish slaughter pigs determined by microbiological testing. *Prev. Vet. Med.* 26:201-213, 1996.

Baggesen, D.L., Wingstrand, A., Carstensen, B., Nielsen, B., Aarestrup, F.M. The effect of tylosin on subclinical infection with *Salmonella enterica* serotype Typhimurium in experimentally infected pigs. *Am J Vet Research*, 60 (10), 1201-1206, 1999.

Baggesen, D.L., Sandvang, D. and Aarestrup, F.M. Characterisation of *Salmonella enterica* serovar Typhimurium DT104 isolated from Denmark and their comparison with isolates from Europe and the USA. *Journal of Clinical Microbiology* 38 (4), 1581-1586, 2000

Sandvang, D., Jensen, L.B., **Baggesen, D.L.**, and Baloda, S.B.: Persistence of a *Salmonella* Typhimurium clone in Danish pig production units and farmhouse environment studied by pulsed field gel electrophoresis (PFGE) *FEMS Microbiology Letters* 187 (1), 21-25, 2000.

Steenhard, N.R., Jensen, T.K., **Baggesen, D.L.**, Roepstorff, A., Møller, K. (2001) Enhanced faecal excretion and mucosal persistence of *Salmonella* Typhimurium in pigs subclinically infected with *Oesophagostomum* spp. *Am J Vet Research*, in press.

CV - Eva Møller Nielsen

Born: 18. September 1962

Education: M.Sc. in Food Technology (1987), Ph.D. in microbiology (1992)

Employment:

1987-88 Research assistant, Dairy Research Institute, Hillerød

1988-91 Ph.D.-student at Dep. Vet. Microbiol., RVAU and Institute of Toxicology, Danish Veterinary and Food Administration.

1991-93 Post-doc at Center for Microbiel Ecology, Uni. of Copenhagen and Inst. of Toxicology

1993-95 Resarch assistant at National Occupational Health Institute, Copenhagen.

1995- Research scientist, and from 1998 senior research scientist, at Danish Veterinary Laboratory, Department of Microbiology, Copenhagen. Primary research activities: Establishment of serotyping system for zoonotic *Campylobacter*, epidemiological *Campylobacter* studies, development of mouse model for studies of *Campylobacter* pathogenesis, zoonotic *E. coli* typing and detection of virulence factors. Surveillance *Campylobacter* in animals, and continuous surveillance of *Campylobacter* serotype distribution animals, food, and humans. Project leader of 'Human diarrhoeagenic *E. coli* in animals and food' (FØSI-001).

Description of role and capacity to provide contribution to the project

Eva Møller Nielsen will be responsible for all laboratory work concerning *Campylobacter*, including method development, analysis and characterisation of isolates.

The laboratory has long experience in *Campylobacter* surveillance and research, i.e. well-established methods for culturing, identification (conventional and PCR-based), serotyping and genotyping. Furthermore, Eva Møller Nielsen is responsible for the addition to the project 'Wildlife as a source of salmonella infection in food-animal production' (FØSI-006), which concerns *Campylobacter* in the production animals and in the wildlife surrounding conventional pig and cattle herds.

Research stays:

1985/86	Food Research Institute, University of Wisconsin, 6 months. Detection of virus in food
1989	BIBRA, England, 2 weeks. In vitro model of human intestinal microflora.
1990	Environmental Protection Agency, North Carolina, 1 week. Methods to investigate survival/effect of genetical modified microorganisms in the gut.
1990	University of Michigan, Ann Arbor, 3 months. In vitro models and mathematical models for the description of the population dynamic in the intestinal microbial ecosystem.
1992	University of Rhode Island, USA, 3 months. Determinants of <i>E.coli</i> and <i>Salmonella</i> to colonize the intestine, lungs and urinary system of mice.
1994	Department of Epidemiologi and Public Health, University of Wageningen. Dr. D. Heederick. Detection of endotoxin, epidemiological methods.
1996	University of Toronto, Canada, 2 weeks. <i>Campylobacter</i> serotyping.

Publications in peer-reviewed journals (total of 27), 5 selected:

Nielsen, E.M., J.Engberg, M. Madsen. 1997. Distribution of serotypes of *Campylobacter jejuni* and *C. coli* from Danish patients, poultry, cattle and swine. FEMS Immunology and Medical Microbiology 19, 47-56.

Nielsen, E.M. & N.L. Nielsen. 1999. Serotypes and tyability of *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry products. International Journal of Food Microbiology 46, 199-205.

Nielsen, E.M., J. Engberg, V. Fussing, L. Petersen, C.-H. Brogren, S.L.W. On. 2000. Evaluation of phenotypic and genotypic methods for subtyping *Campylobacter jejuni* isolates from humans, poultry, and cattle. Journal of Clinical Microbiology 38, 3800-3810.

Petersen, L., **E.M. Nielsen**, J. Engberg, S.L.W. On, H.H. Dietz. 2001. Comparison of genotypes and serotypes of *Campylobacter jejuni* from Danish wild mammals and birds and from broiler flocks and humans. Applied and Environmental Microbiology, 67, 3115-3121.

Nielsen, E.M. J. Engberg, V. Fussing. 2001. Genotypic and serotypic stability of *Campylobacter jejuni* strains during in vitro and in vivo passage. International Journal of Medical Microbiology 291,379-85.