Final report

for

Project no. 1888

Risks and Recommendations Regarding Human Pathogens in Organic Vegetable Production Chains (PathOrganic)

Period covered: **Final:** **01.08.2007 – 31.07.2011**

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CORE Organic

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Coordinator information:				
Institution:	AIT Austrian Institute of Technology GmbH		Acronym AIT	
Faculty/ Department/ Section/Unit	Health & Environment Bioresources Unit			
Address:	Road name and number: Konrad Lorenz Straße 24			P.O. Box:
	Town Tulln	Post Code: 3430	Region:	Country: Austria
Coordinator:	Family name: Sessitsch	First name: Angela	Title: Dr.	
Address if different from above:				
	Phone: ++43(0)50550 3509	Fax: ++43(0)50550 3666	E-mail: Angela.sessitsch@ait.ac.at	
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Project partners and contact persons:

Partner no.	Country	Organisation name:	Functions*):	Involved in WPs:	Contact person:
1	**AT**	**AIT**	PC, WPM (1), WPCM (4)	1, 2, 3, 4	Angela Sessitsch
2	**AT**	University of Natural Resources and Life Sciences Vienna (BOKU), Department of Sustainable Agricultural Systems	P	1, 2, 3, 4	Jürgen K. Friedel
3	**CH**	Research Institute of Organic Agriculture (FiBL)	WPM (4)	1, 2, 3, 4	Gabriela Wyss/ Martin Koller
4	**CH**	Agroscope Changins-Wädenswil (ACW)	WPM (2)	2, 3, 4	Brion Duffy
5	**CH**	Agroscope Reckenholz-Tänikon (ART)	P	1, 2, 3, 4	Franco Widmer
6	**DE**	Helmholtz Zentrum München Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH)	P	1, 2, 3, 4	Anton Hartmann
7	**DK**	Technical University of Denmark National Food Institute (DTU-Food)	P	1, 2, 3, 4	Dorte Baggesen
8	**DK**	University of Copenhagen Faculty of Life Sciences (CU-LIFE)	P	1, 2, 4	Anders Dalsgaard
9	**NL**	Plant Research International B.V. (PRI)	P	1, 4	Carolien Zijlstra
10	**NL**	Wageningen University (WUR)	P	1, 4	Ariena van Bruggen
11	**SE**	Swedish University of Agricultural Sciences (SLU)	WPM (WP 3)	1, 2, 3, 4	Veronica Arthurson

***)** PC: Project Coordinator, WPM: Workpackage Manager, WPCM: Workpackage Co-manager, P: Participant

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Project Summary, including objectives and expected outputs

(to be taken from original proposal (max. 1 pg. - Arial, size 11)

PathOrganic assesses risks associated with the consumption of fresh and minimally processed vegetables due to the prevalence of bacterial human pathogens (e.g. *Salmonella enterica*, pathogenic *E. coli*, *Campylobacter* spp., *Listeria monocytogenes*) in organically grown plant produce. The project aims at evaluating whether organic production poses a risk on food safety and addresses the food chain by taking into consideration potential sources of pathogen transmission (e.g. animal manure). In addition, it evaluates whether organic production may reduce the risk of pathogen manifestation.

From a European perspective, vegetable-linked outbreaks are not well investigated. Within the PathOrganic project, surveys of organically grown vegetables are carried out in five European countries, and regionally different environmental and management factors are taken into consideration for performing risk assessment.

In order to carry out a meaningful survey and to choose appropriate experimental plans, a model for pathogen transfer to selected plants is built in work package 1 (WP 1) to describe relevant food chains. Based on this model sampling strategies and methodological adjustments are made. A questionnaire is set up to describe current management practices (WP 1). WP 2 of the project is dedicated to surveying the presence of food pathogens in organic plant produce. As a major part of the project, field surveys of organic farming systems are performed and vegetable plants are sampled in five European countries. The analysis of relevant parameters is done by the respective partners with the appropriate expertise. “Risk crops” and “risk factors” are identified and specific production procedures shall be investigated, leading to the determination of critical control points (CCPs). Factors suggesting a problem concerning food safety are subjected to more detailed analysis in WP3 by performing specifically targeted greenhouse and field experiments. These experiments allow analyzing critical environmental and management factors and at the same time enable to re-assess the critical control points. Finally, based on the results obtained in the previous WPs, in WP 4 recommendations will be provided for improving procedures which secure consumer-oriented food safety and the quality of certified organic vegetable food chains. Thus, project results shall enable producers and other stake holders to prevent and better control plant produce-associated outbreaks.

1. Main results, conclusions and fulfillment of objectives

1.1 Summary of main results and conclusions

The project examined routes of pathogen transmission, in particular the transfer via animal manures used for fertilization, and evaluated whether commonly used organic production practices pose a risk on food safety. On the other hand, the project also analyzed whether organic production may reduce the risk of pathogen manifestation due to improved soil buffering.

In work package (WP) 1, input from all PathOrganic partners relating to the current practice in organic vegetable production in the individual countries was assembled for developing a questionnaire by the FiBL Swiss partner institute. Besides contributing to the construction of the questionnaire by the FiBL partner, this information allowed to select for organic vegetable farms where screenings of organic fertilizers and lettuce plants were performed in WP2. Two further questionnaires were used to collect detailed information needed for the risk assessment procedure.

Screenings of animal manures were accomplished by collecting in total 151 manure and slurry samples that were used as fertilizers at organic vegetable farms. Sampling was done according to a strategy that was jointly elaborated by the PathOrganic partners. Regarding microbiological analyses, each lab was in charge of analyzing the collective samples from all countries for the prevalence of a specific pathogen. Common protocols were used in all labs for the preparation of the enrichment cultures and for successive DNA isolation to allow for standardization of analysis procedures. Most commonly, the shiga-toxin producing *E. coli* (STEC) virulence genes *stx2*, *stx1*, and *eae* intimin were found in the manure samples, which prevailed in 57, 45, and 70% of all analyzed samples, respectively. The *rfbE* gene, specific for enterohaemorragic *E. coli* O157:H7 (EHEC), was found in 34% of the samples. The corresponding findings of *S. aureus*, *Salmonella* spp., *Campylobacter* spp., and *L. monocytogenes* were 17, 11, 9, and 8% respectively. Also, the chemical properties and bacterial community structures of the samples were investigated and possible correlations between all these parameters were studied. Most correlations with chemical parameters were found for *stx1*, which was positively correlated with dissolved organic carbon and carbon content, and negatively correlated to phosphorus and nitrate concentrations. Bacterial community structures differed between some of the countries with most similar structures in Austria, Switzerland, and Germany.

The screenings of organic fertilizers from a range of vegetable farms represented the basis for successive screenings of vegetables grown on farms where a potential safety hazard was indicated. Analyses were concentrated on leafy vegetables such as lettuce and spinach, because here fertilization practices were most likely to cause pathogen transfer to the plants, and additionally included carrot samples. The vegetable samples analyzed comprised 1973 lettuce heads (romaine, iceberg, and butter head), 190 spinach plants, 50 carrots, and 50 corn salad plants. All samples were processed in the same way in the various labs participating in the screening for the preparation of DNA extracts and subsequent analysis for pathogenic bacteria. Our study revealed that an unexpected high number of vegetables collected from the selected fields were colonized by bacterial pathogens. As enrichment was used prior to PCR-based analysis, the screening results may not pose an implicit threat to the consumer due to possibly low pathogen numbers. Yet, our results stress the need for stringent post-harvest handling and processing procedures in terms of restricting survival and proliferation of pathogens possibly introduced on the field.

In WP3, greenhouse/growth chamber/phytotron experiments were carried out to study in detail which management factors may lead to the contamination of organically grown vegetables with human pathogens. The experiments centered on the colonization behavior of various bacterial pathogens in the plant and in the soil, and on specific effects of the plant species and genotype. In addition, we studied effects of fertilizer management on the pathogen translocation from manures and slurries and explored biological buffering by the indigenous soil microbial communities.

Critical Control Points (CCPs) were evaluated for organic farms that use manure for the production of organic lettuce, cabbage, carrots and spinach in Austria, Switzerland, Sweden, and Denmark.

The hazard analysis was based on the characteristics of the enteropathogens analyzed within the project and on the agricultural practices applied by the farmers. Assessment of the actual agricultural practices was done by interviewing 16 farmers in Austria, 16 farmers in Switzerland, 13 farmers in Sweden, and 9 farmers in Denmark.

Another project result is the development of a conceptual model that quantifies the inactivation and proliferation of pathogens in the pathway manure – soil – crop. Experts within the project were asked to provide input, based on the knowledge obtained in the PathOrganic project, specifically data on persistence in the soil and the concentrations of specific pathogens needed to get infection/contamination of the crops. Still, a bottleneck remains regarding the data on manure storage times, fertilizing practices and realistic initial concentrations of pathogens that reflect current practices for different crops / pathogens/ soil types. A project workshop was organized, in which critical control points were discussed and recommendations were formulated. As a major deliverable of the project, the Swiss project partners produced a leaflet containing recommendations concerning safe production procedures. Thus, the project results allow farmers and food processing companies to better control microbe-mediated contamination of organically grown vegetables, and thereby may help support organic production and sustainable agriculture.

1.2 Fulfillment of objectives

To what extend did the project achieve its objectives?

Major objectives of the project were to investigate pathways of pathogen infestation of vegetables, to examine in detail which factors contribute to contamination, and, finally, to provide recommendations for safe vegetable production procedures.

A basic problem encountered was that vegetable production procedures varied considerably among the participating countries, and that contamination pathways along the production line appeared to be highly complex. To nevertheless be able to satisfactorily address the project tasks, we concentrated on animal manures as a major source of pathogen infestation on the field. Thus, more effort than originally planned was spent on investigating current production practices (by using questionnaires), and more extensive screenings of manures and vegetables on the field were carried out.

The surveys and experiments yielded a multitude of data, which were valuable for elucidating mechanisms of pathogen transfer to vegetables on the field but, however, were not appropriate for building the quantitative microbiological risk assessment (QMRA) model that was initially anticipated. This led to a necessary change in the model strategy. Therefore, we developed a conceptual model that quantifies the inactivation and proliferation of pathogens in the pathway manure – soil – crop.

Based on the survey results and the results of specifically designed experiments performed in the greenhouse, critical control points were identified and recommendations were formulated for farming practices that reduce risks of pathogen infestation. Thus, the final project objectives were achieved. Conclusions and recommendations were presented to the public and to stakeholders during a stakeholder workshop, and were disseminated (among others) via a leaflet.

2. Milestones and Deliverables status

Milestones:

Milestone no:	WP	Description	Planned time	Actual time
1i	**1**	Extended model outline for pathogen transfer through use of manure-based fertilizer	month 6	month 6
1ii	**1**	methods adapted for the detection of pathogens	month 10	month 7
1iii	**1**	protocols established for sampling procedures and surveys	month 12	month 7/ month 10
2i	**2**	identifying high-risk crops and production systems	month 16	month 17
2ii	**2**	assessing which data are available or lacking for performing MRA	month 28	month 28
2iii	**2**	description of food chains and listing of critical control points in the production chains	month 28	month 32
3i	**3**	identify susceptible plant species and genotypes	month 45	month 45
3ii	**3**	produce additional and necessary data for MRA	month 44	month 45
3iii	**3**	determine the effect of biological buffering on pathogen persistence	month 45	month 45
3iv	**3**	identify risk factors in experimental trials	month 45	month 45
4i	**4**	Refining the conceptual model by integrating experimental data	month 44	month 45
4ii	**4**	recommendations for improved farm management procedures	month 30	month 32
4iii	**4**	a workshop to evaluate the risk model and discuss the critical control points and recommendations	month 33	month 32
4v	**4**	non-scientific and scientific publications for end-users (e.g. consumers and science)	month 48	month 48
4vi	**4**	reporting to the EU and national programs	month 48	month 48

Deliverables:

Deliverable no:		WP	Description	Planned time	Actual time
1a	**O**	**1**	Lab-workshop among project participants on method harmonization	month 5	month 5
1b	**O**	**1**	Protocols on methods for the detection of pathogens in complex biological matrices	month 10	month 7
1c	**O**	**1**	Final protocols for sampling and surveying developed	month 12	month 7 (manures)/ month 10 (vegetables)
1d	**O**	**1**	Conceptual model on pathogen transfer developed	month 28	month 32
2a	**O**	**2**	Information to farmers (leaflets)	month 7	month 7
2b	**C**	**2**	Presentation of project (in particular WP2 results) at international conferences	month 18	months 13 to 18
3	**C**	**3**	Presentation of project (WP2 and WP3 results) at international conferences	month 38	month 24
4a	**O**	**4**	recommendations for improved farm management procedures (leaflet or information on web page)	month 44	month 45
4b	**O**	4	Final workshop	month 33	month 32
4c	**S**	4	Risk assessment model on pathogen transfer developed based on the program “@Risk” presented in a scientific publication	month 44	month 45
4d	**S/P**	4	non-scientific and scientific publications prepared based on WP2 and WP 3 results	month 48	month 48
4e	**R**	4	reporting to the EU and national programs	month 48	month 48

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

1iv	**1**	Development of a conceptual model based on evaluation of questionnaires	month 28	month 32

has been moved to

4i	**4**	Refining the conceptual model by integrating experimental data	month 44	

3. Work package description and results:

WP 1	Current practice and harmonization of methods
Responsible partner: 1, AIT, Angela Sessitsch	
Description of work: (In WP 1 a conceptual model for pathogen transfer to selected plants is built based on the investigation of current practices of manure-derived fertilizer application in organic plant production (DTU-Food/CU-LIFE). The model takes into consideration nationally different manure application practices and will be the basis for the final microbiological risk assessment model (to be developed in WP4). In the individual countries, current practices of manure derived fertilizer application are inquired and protocols are developed for sampling and for the application of microbiological techniques for pathogen detection and quantification in complex biological matrices. Questionnaires are set up by FiBL including input from all partners concerning local requirements. The following **tasks** are addressed in WP 1: **i)** development of a model describing routes of pathogen transfer; **ii)** optimization of pathogen detection methods; **iii)** exploration of food-production chains by the use of questionnaires; **iv)** development of survey protocols.	

Final report on work carried out and results compared to the original plan/WP aims:

A- work carried out and results obtained

Ad Task 1i (model development)

An outline of all possible pathogen transfer routes in organic vegetable production was prepared based on the Dutch *E. coli* O157 @Risk model by Franz et al., which was presented at the 4th PathOrganic meeting in March 2009. Points of concern raised by this outline were included in the comprehensive questionnaire (**Q3**, WP1) regarding manure practices in organic farming. Additionally, issues pin-pointed by the outline that concerned the ability of human pathogens to colonize leafy crops were addressed in WP3. Common farm management practice and available data differed considerably between participating countries, so that these differences first had to be elucidated before the conceptual model planned within WP1 could be fully established. Hence, since further analysis of questionnaires was required to build the conceptual model, this deliverable was shifted to WP4.

Ad Task 1ii (detection methods)

Expertise from the individual labs was used to jointly develop protocols for sample preparation, preparation of enrichment cultures, DNA isolation and chemical analyses. At the individual labs, microbiological protocols were specifically adapted to the analysis of manures and vegetable samples, with each lab specializing on the analysis of a specific pathogen. These protocols were tested and finally approved of by the participating labs during a lab-workshop that was held subsequent to the second PathOrganic Consortium Meeting in Munich in January 2008.

Contributions by the individual partners:

- DTU-Food and to some extent CU-LIFE provided information on the standard (ISO) methods for detection and quantification of *E. coli.*
- PRI provided information regarding protocols for DNA extraction and PCR.
- By SLU PCR primers and proper PCR programs were evaluated and optimized for the detection of *Campylobacter* spp. (jejuni/coli) in soil, manure and plant produce, with emphasis on specificity, sensitivity (detection limit), and speed.

- HMGU developed a detection method for *Listeria monocytogenes* including the appropriate enrichment procedure and a DNA isolation protocol.
- AIT developed protocols for ISO- and PCR-based analysis of manure and vegetable samples for *Staphylococcus aureus*., including the appropriate enrichment method.
- ACW developed protocols for *Salmonella* sp. and *E. coli* O157:H7 enrichment from manure and vegetable samples and ISO- and PCR-based detection methods.

Ad Task 1iii (questionnaires)

A first **questionnaire** (**country questionnaire, Q1** (see Addendum II) was set up by FiBL by mid September 07 to facilitate the decision making process on the selection of vegetable crops and manure types to be included in a survey on possible pathogen contamination in the organic vegetable production chain. In the individual countries, information asked for in the questionnaire was supplied by farmers´ associations (e.g. Bio-Austria in Austria, www.bio-austria.at) and by local experts. The completed questionnaires were evaluated by FiBL and served as a basis for drafting adequate sampling protocols, which were presented during the second project meeting in Munich in January 2008.
Evaluation of Q1 gave the following results:
a) Herbs and vegetable crops:

- Selection for vegetables e.g.
  - Same crop(s) in all countries
  - Country specific survey
  - One common crop and one crop typical for the country
- Vegetables types to choose from
  - Root vegetables: Carrots, onions
  - Above ground: Cabbage, Brassicaceae
  - Above ground / leafy vegetables: Spinach, lettuce
  - Greenhouse production
- Manure sampling prior to plant sampling to define risky crops

b) Manures:
Fertilization application generally diverse

- Small-scale farms (traditional, less intense) often include animal husbandry (beef cattle)
  - Preferably cattle manure (organic source)
  - Preferably solid manure, farmyard manure
  - Supply from mixed sources
  - Cattle > pig > chicken
- Large-scale farms (intensive vegetable production)
  - Preferably pig manure
  - Preferably liquid manure
  - Supply preferably from conventional/mixed sources
  - Pig > chicken = cattle
- Manure application in conventional agriculture in A, CH, DK

Conclusions for **sampling procedures:**
Plants: Carrots seemed to be an important raw-eaten vegetable and therefore should be included in the survey. In addition, AT, D and CH also addressed spinach, whereas the second crop in SE and DK was decided to be lettuce. Later on, it was decided against sampling carrots in Austria, Germany and Switzerland because of restrictive fertilization practices used as a measure of pest control.
Manure: It was decided that manure samples should be taken at the farm on which the manure was produced in the same condition as it is generally used for fertilization. There should be ideally an equal distribution of pig slurry, cattle slurry and cattle manure as well as an equal distribution of conventional and organic-derived manure. However, in reality the distribution might look differently.

With the aim to collect background information for the manure screenings, a questionnaire to be completed at the samplings was developed by FiBL for the use by all partners (**Q2**, see Addendum II). The Questionnaire was translated into the various languages by the individual partners.
Results from questionnaire Q2 were used to study relationships between pathogens occurrence and chemical parameters. Based on the results from Q2, a **comprehensive questionnaire (Q3)** on management practices at farm level in relation with crop management, manure application practice (animal manure, compost, biogas slurry, mulch), harvest, and storage was elaborated. After testing by an adviser the questionnaire was sent out in spring 2009. The results were filed into a database and were used to determine critical control points (CCP) in WP 4.

The timing of manure application is especially critical for pathogen survival and therefore for potential risks. According to questionnaire Q2 entries, 64 to 70% of the farms used manure "directly before" planting carrots, lettuce, spinach or brassica crops (range from several days to two months; Fig. 1). One farmer used slurry after spinach establishment; however, this produce was used for processing purposes and the product was intended for cooking. In carrot (n=2) and brassica crop cultivation, two respectively five farmers used manure after crop establishment. However, these crops normally take more than 100 days from planting to harvest.

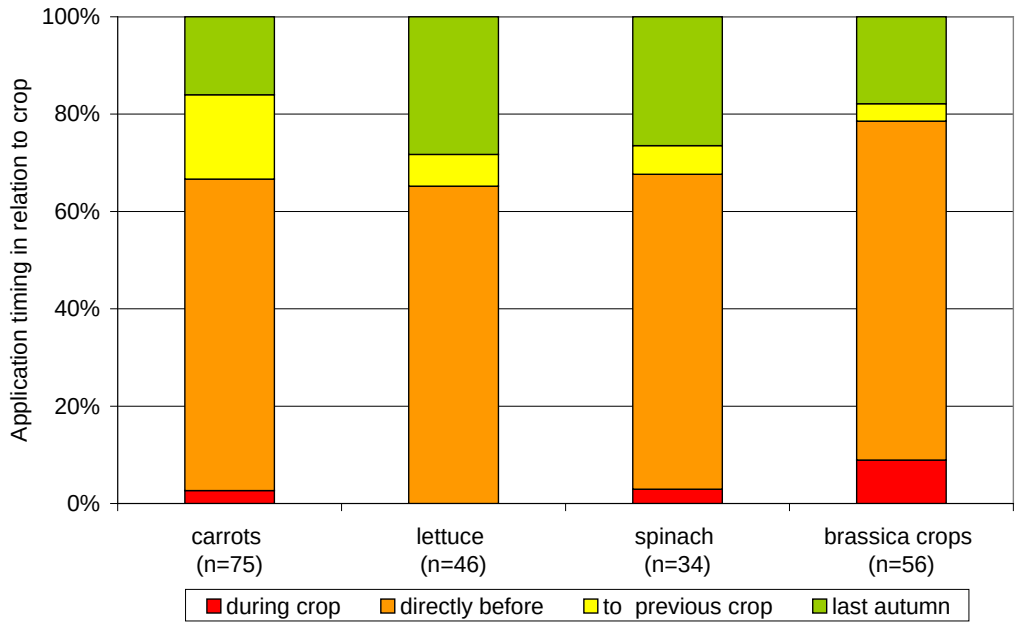

Figure 1. Timing of manure application on different farms in Austria, Germany, Denmark, Sweden and Switzerland according to questionnaire Q2 entries.

Ad Task 1iv (survey protocols)

A common strategy for the sampling of organic fertilizers (manure, slurry and composted manure) was jointly elaborated by the PathOrganic partners. Besides sampling fertilizers and vegetables, it was agreed to collect information from the farms referring to the type, origin (animal, conventional /organic), storage and treatment of the fertilizers, to feeding practices of the livestock and potential feeding additives as well as to fertilization practices by use of a questionnaire (Q2, see above and Addendum II) developed by the FiBL partner.
Similar to the fertilizer screenings, a common strategy was followed by all project partners concerning the sampling of vegetables from fields where results from fertilizer analyses indicated a potential risk of contamination with human pathogens. This strategy was aimed at collecting 500 plants of lettuce, spinach or carrot from each of the respective fields. Specific survey protocols that were developed by the individual partners are given in 2ii.

Contributions by the individual partners:

- FiBL and BOKU jointly developed a protocol for the sampling and sample preparation of animal manures. In addition, they elaborated protocols for chemical analysis of manure samples.
- HMGU developed a survey protocol for the sampling and sample preparation of carrot.
- ARC developed a survey protocol for the sampling and sample preparation of lettuce.
- ACW developed a survey protocol for the sampling and sample preparation of spinach.

B- comments on deviations from the original plan

The model outline for pathogen (*Escherichia coli* O157) transfer through the use of manure-based fertilizer (dairy manure) by Franz et al. (2008) needed an extension to cover the major differences in common farming practice as revealed by the initial country questionnaires (Q1). Thus, more effort than originally planned was put into data exploration, and task 1iii (questionnaires) was intensified. The extended outline model helped in constructing the comprehensive questionnaire (Q3, WP1).
Model extension by the Danish partner was delayed as compared to the original plan because of major differences in common farming practice and available data among the participating countries. Gradually, specific data became available from WP 3 and WP 4 experiments, which were used for the final adjustment of the risk assessment model developed in WP 4 (month 35). Thus, there were no delays in the activities planned for the remaining project period. We have provided an adjusted time plan for the model development (see deliverables and milestones lists).

Protocols for sampling, sample preparation and pathogen detection in complex biological matrices have been developed by the individual consortium partners even earlier than set in the time-plan, and protocols were jointly evaluated and approved of during a lab-workshop held subsequent to the second PathOrganic Consortium Meeting in Munich.

WP 2 Surveys of food-borne pathogens

Responsible partner: 4, ACW, Brion Duffy

Description of work:
The aim of WP 2 is to identify high risk-vegetables in organic production and to identify potential exacerbating factors. Incidence and levels of enteric pathogens (pathogenic *E. coli*, various serovars of *S. enterica*, *L. monocytogenes*, *C. jejeuni*, and *S. aureus*) associated with selected vegetables are determined and the prevalence of pathogens in diverse amendments (e.g. manure, compost, slurry) used in organic production systems are assessed to pin-point potential sources of contamination.
WP 2 comprises the following **tasks**: **i)** literature study; **ii)** survey of organic plant produce in the field; **iii)** identification of critical control points in the entire production and food chains; **iv)** determination of information gaps in relation to MRA.

A- work carried out and results obtained

Ad Task 2i (literature study)

This task was carried out by all project partners in order to develop a common strategy for the manure and vegetable screenings (see below). Support in strategic planning was provided by PRI-WUR through presenting to the project partners the work on enteric pathogens done at Wageningen University.

Ad Task 2ii (field surveys)

Screening strategy

Organic fertilizers have been identified as a major risk factor potentially contributing to the contamination of vegetables with human pathogens. Therefore, manures, slurries and composted manures that were commonly used for fertilization at the selected farms were analyzed for the prevalence of human pathogens most commonly associated with vegetable contamination. In addition, analyses of chemical properties of the manures were done in the individual countries following common protocols. Arrangements were made for chemical analyses of manure and slurry samples from Denmark at Wageningen University by the Dutch partner.
The screenings of organic fertilizers from a range of vegetable farms in five different countries represented the basis for successive screenings of vegetables grown on the respective fields. Hence, those farms were selected for screenings of vegetables where a potential safety hazard was indicated by the prevalence of human pathogens in manure or slurry samples.
According to a joint strategy developed by the PathOrganic partners, each lab was in charge of analyzing the collective samples from all countries for the prevalence of a specific pathogen. Common protocols were used in all labs for the preparation of the enrichment cultures and for successive DNA isolation, and DNA preparations were then sent via mail to the different labs for PCR-based screenings for specific pathogens. This procedure allowed for standardization and granted comparability of results. Besides molecular analyses, cultivation-dependent ISO-analyses were carried out for testing the samples from the individual countries for the specific pathogen that was also targeted by PCR-based analysis in the respective lab. An overview of the PathOrganic strategy for the sampling and analysis of manures and vegetables is given in Figures 2 and 3.

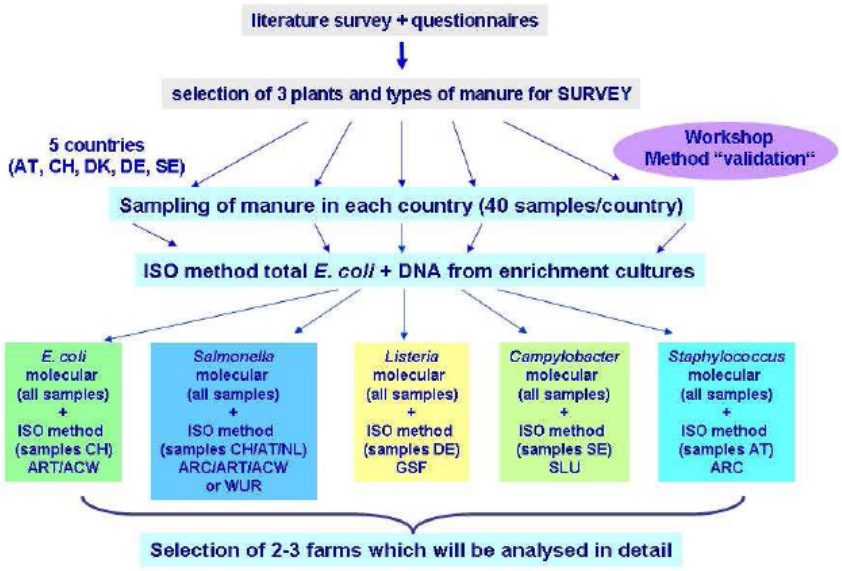

Fig. 2. General scheme of the PathOrganic sampling and analysis strategy used for the manure screenings.

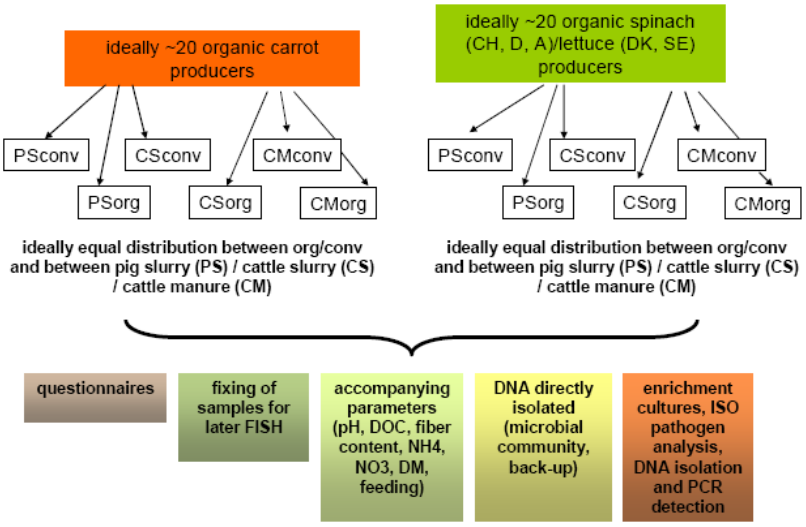

Fig. 3. Scheme of the PathOrganic strategy used for manure and vegetable screenings indicating analysis procedures as well as selection of vegetable and manure types.

Sampling protocols for carrot (HMGU) and for lettuce and spinach (AIT and ACW) are provided in the deliverables folder.
Protocols for chemical analysis of manures and protocols for pathogen analyses in manures and vegetables are given in ANNEX3.

Screening of Organic Fertilizers for Human Pathogens

BOKU and AIT jointly collected manure and slurry samples from 40 farms, which were located in different geographical regions of Austria, during March and April 2008. FiBL collected 40 manure and slurry samples based on the elaborated sampling procedures during spring 2008. FiBL and BOKU provided protocols for measuring the chemical properties of manure samples. In Denmark, a total of 34 manure samples representing 24 vegetable producers were sampled in Spring 2008. By SLU, 28 manure samples were collected from different farmers. The manure analyzed was used at 20 carrot farms and 20 lettuce farms in total. By HMGU, 9 manure samples were collected and processed according to the developed protocols.

In the individual labs, enrichment cultures were prepared from the manure samples by following a common protocol, and microbial DNA was isolated for subsequent analysis of the samples for the prevalence of pathogenic bacteria. The various labs were in charge of analyzing the collective samples from all countries for the prevalence of a specific pathogen. Thus, at the AIT lab PCR-based analysis for the detection of *Staphylococcus aureus* was performed on DNA preparations from enrichment cultures from all countries. PCR-based analyses of fertilizers for *Salmonella* sp. were shared among AIT and ACW, with AIT being responsible for the analysis of samples from Austria, Sweden and Denmark, while ACW was in charge of PCR-based analysis of samples from Switzerland and Germany. ACW performed analyses of the collective samples for the prevalence of pathogenic *E. coli* by testing the samples for *rbfE* genes indicative of *E. coli* O157, as well as for *stx*1, *stx*2 and *eaeA* virulence genes. By SLU analysis of the collective samples for Campylobacter was done, and HMGU performed analyses for *Listeria sp*. Besides molecular analyses, cultivation-dependent ISO-analyses were carried out in the labs where the respective PCR-based methods were applied. Thus, Austrian samples were analyzed for the prevalence of *S. aureus*, while Swiss samples were tested for *E. coli* O157:H7 and *Salmonella* sp., Swedish samples for *Campylobacter* spp. and German samples for *Listeria sp*. Results of the screenings of manures are presented in a

publication by Jäderlund et al. (submitted to Applied & Environmental Microbiology)
Comparison of results obtained by ISO and PCR-based methods revealed a higher number of positive samples by plating versus PCR for the detection of *Staphylococcus* sp., because the ISO method was responsive to the presence of a broader range of staphylococci. By contrast, the PCR-based method specifically targeted *S. aureus.* In a similar way, the ISO-method for detecting *Listeria* sp. seemed to be less specific than the PCR-based method. *Campylobacter* ISO and PCR-based methods gave corresponding results, while low viability of *E. coli* cells in the samples probably resulted in negative ISO-values relative to the PCR measurements. Based on the results obtained in the manure screenings it was decided to use only the PCR-methods in subsequent screenings of vegetables.

Data from questionnaire Q2 and results from chemical analysis of manures and slurries proved useful for interpreting the findings of the manure survey. Manures were grouped based on data collected via the questionnaire. Stable manures (stacked and deep litter) showed a pronounced anaerobic pattern, and rotted manures were also dominated by anaerobic processes. Composted manure that is frequently turned typically showed aerobic reactions such as nitrification.
Slurry samples contained the highest numbers of pathogens and virulence numbers, with exception of *Staphylococcus aureus*, (Fig. 3). By contrast, composted manure showed significantly lower numbers of pathogens and virulence factors than stable manure and slurry. This corresponds with a higher nitrate to ammonia ratio and lower content of dissolved carbon (DOC) and nitrogen (DN,). Nitrification is an obligatory aerobic reaction, and therefore a high nitrate to ammonia ration is an indicator of aerobic conditions. Dissolved carbon (DOC) and dissolved nitrogen (DN) are easily available nutrients (Table 1).

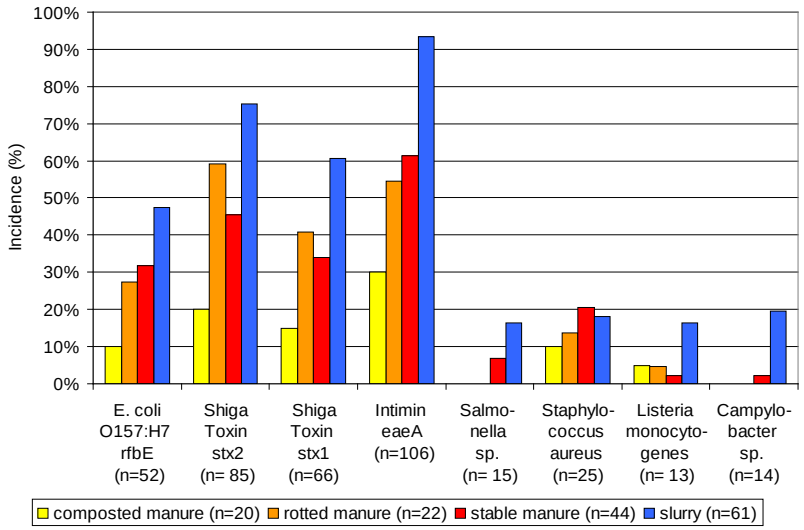

Figure 3: Incidence of pathogens/virulence factors in different manures or slurries (without fermented slurry, because of small samples numbers)

Table 1. Influence of manure type on pathogens & virulence factors and chemical parameters (Means and standard deviation)

Type of manure	N	pathogens & virulence factors (1)	Dry matter (%)	NH_4-N mg x g^{-1} DM	NO_3-N / NH_4-N	Dissolved carbon (mg x g^{-1})	Dissolved nitrogen (mg x g^{-1})
composted manure	20	0.9 ± 1.4 c (2)	28.2 ± 16 a	1.3 ± 3 c	15.5 ± 32 a	20.9 ± 8 b	13.2 ± 19 b
rotted manure	21	1.9 ± 1.0 b	25.0 ± 15 a	4.7 ± 6 b	5.6 ± 12 a	26.4 ± 10 b	18.7 ± 20 b
stable manure	44	2.1 ± 1.7 b	25.3 ± 14 a	5.8 ± 15 b	6.1 ± 14 a	25.9 ± 16 b	23.9 ± 32 b
slurry	60	3.5 ± 1.4 a	4.6 ± 6 b	26.8 ± 29 a	1.3 ± 6 b	52.4 ± 39 a	64.0 ± 61 a
Kruskal-Wallis		p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001

(1) Numbers of pathogens and virulence factors per manure sample (mean per manure type)
(2) Nemenyi (5 %) *a posteriori* test after non-parametric Kruskal-Wallis test. Samples (original or transformed) were not normal distributed or homogeneity of variances was not sufficient. Different letters within columns indicate that values are significantly different.

Screening of Vegetables for Human Pathogens

By HMGU, 50 carrot samples fertilized with manure G01MC00 (25 samples) and manure G02SC00 (25 samples), 50 spinach samples fertilized with manure G01MC00 (25 samples) and manure G02SC00(25 samples), 40 Spinach samples fertilized with manure G08SC00, and 50 corn salad samples fertilized with manure G08SC00 were collected for analysis.
BOKU and AIT collected 500 plants of green lettuce from field A30MK, 350 plants of iceberg lettuce from field A34MP; and 300 plants in total of green lettuce, endive and Lollo Rosso lettuce were collected from field A17MP.
By SLU three lettuce farms were picked for vegetable sampling based on the prevalence of human pathogenic bacteria in manure samples. At each farm, 50 samples of 10 plants were collected, in total 500 plants were collected at each farm.
In Denmark, three farms were selected for harvest of 50, 50 and 47 samples of lettuce (10 heads each), respectively.
1000 spinach samples were collected from 2 fields by ACW and FiBL.

In the vegetable screenings only PCR-based methods were used due to higher specificity as compared to cultivation-dependent analysis. Analysis of the collective DNA-samples prepared from enrichment samples was done in the individual labs according to the same scheme as was followed in the manure screenings, except that analysis for *Salmonella* sp. was done by AIT for all samples.

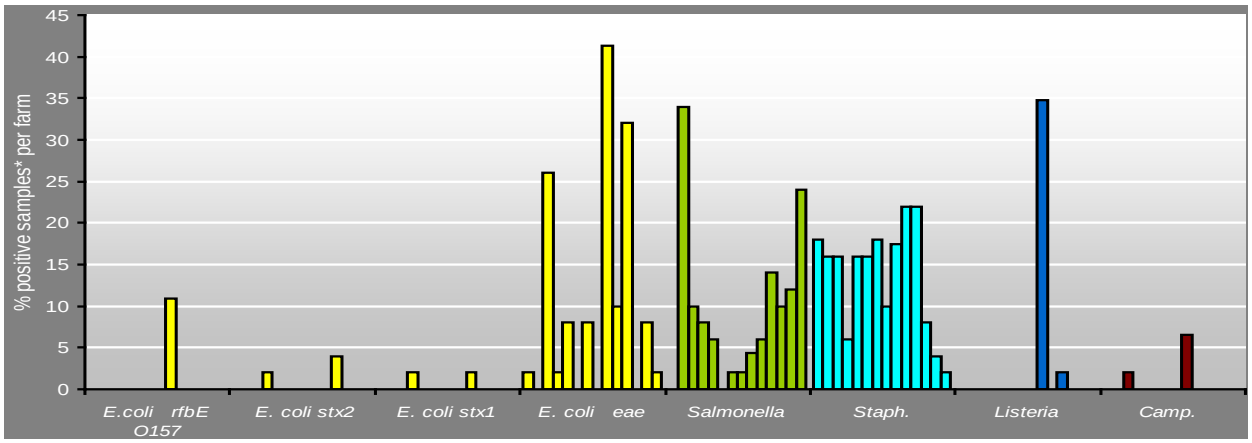

Figure 4: Incidence of pathogens/virulence factors in vegetables collected from 14 farms. 1 sample = 25 g of inner and outer leaves from a batch of ~10 spinach or lettuce plants/ 25 g from 10 carrots

Results from the vegetable screenings are presented in a publication by Fenzl et al. (submitted in FEMS letters).

Ad Task 2iii (critical control points) and Task 2vi (MRA missing information)

Evaluation of both questionnaire and survey results revealed which data were missing for determining the main factors that are critical for avoiding fresh produce contamination with human pathogens.

First of all, two entrance points for the bacteria into the plant seemed plausible; via the surface area or via the roots. However, it was shown that it was very hard to estimate the numbers of bacteria required in manure-amended soil to 'contaminate' a plant due to the complexity of factors of potential importance for such contamination. It appeared that for data to be of relevance they should reflect what is going on under natural conditions. Secondly, quantitative data on the fate of bacteria in/on the plants was limited and an essential question was whether the bacteria would die off or if proliferation can occur, as this will influence the final contamination risk significantly. Thus,

greenhouse and field experiments (WP3) were specifically designed to gain additional information.

Critical control points that were identified based on questionnaire and survey results were re-assessed in the WP3 experiments.

Critical Control Points (CCPs) were evaluated for organic farms that use manure for the production of organic lettuce, cabbage, carrots and spinach in Austria, Switzerland, Sweden, and Denmark. This work relied partly on the principles and steps described for HACCPs in the course guidance document "HACCP in agriculture – Organic milk production" (van Veluw et al., 2008). CCPs were identified by using the decision tree of the HACCP system. Where appropriate, PRP-CPs (Prerequisite Programme Control Point; Control Point covered by a Prerequisite Programme) or OP-PRPs (Point already covered by an Operational Prerequisite Programme) were defined instead of CCPs. This has been formulated in the report "PathOrganic - Identification of Critical Control Points for organic vegetable crops" as one of the project's deliverables.

The hazard analysis was based on the characteristics of the enteropathogens analyzed within the project and on the agricultural practices applied by the farmers. Assessment of the actual agricultural practices was done by interviewing 16 farmers in Austria, 16 farmers in Switzerland, 13 farmers in Sweden, and 9 farmers in Denmark. In general, there was no agricultural practice common to the majority of organic farmers concerning the management of animal manure, fertilization, irrigation, harvest and postharvest management when growing lettuce, cabbage, spinach, and carrots in Austria, Switzerland, Denmark, and Sweden.

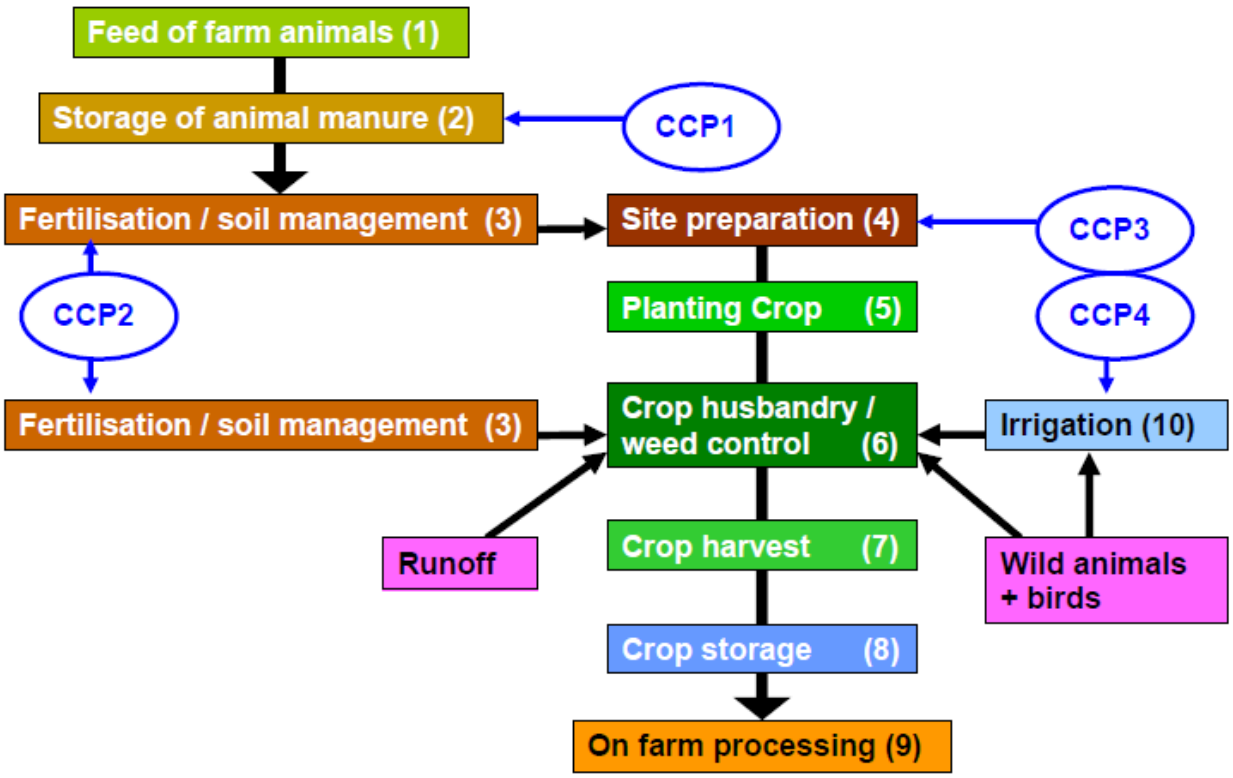

Figure 4: Critical control points identified in steps of field vegetable production.

References

Van Veluw, K., F. Bodnar, C. Knight, R. Stanley, and C. Leifert (2008): HACCP in agriculture – Organic milk production. Course guidance document.

B-comments on deviations from the original plan

Higher numbers of manure and vegetable samples were analyzed than previously anticipated because of the decision taken by the PathOrganic consortium to concentrate on the first step in the production line, involving potential pathogen infestation of vegetables via manure application.

WP 3	**Mechanistic description of food contamination with human pathogens**

Responsible partner: 11, SLU Veronica Arthurson; 1, AIT, Angela Sessitsch

Description of work:

The aim of WP3 is to study those factors in detail which potentially lead to the contamination of organic plant produce with human pathogens. Factors which in WP2 have been implied to be problematic are subjected to more detailed analysis so that risk mitigation strategies may be developed. Interactions between microbial strains representing major food pathogens and various plant cultivars used in organic farming are studied in detail, because different plant species and genotypes usually interact very specifically with microorganisms. In particular, research within WP3 addresses pathogen transfer and survival in association with multiple modes of usage of animal-derived fertilizers. Greenhouse/growth chamber/phytotron experiments are carried out at several participating laboratories using soils from various European long-term organic field experiments in different geographic regions. The persistence in soil and plant uptake of human pathogens which have been applied to manure in different concentrations are studied in detail. Based on the information from WPs 1 to 3 a quantitative microbiological risk assessment is performed by the Danish project partner.

WP3 is divided into the following **tasks**: **i)** testing the effect of plant genotype on pathogen colonization; **ii)** testing the effect of the management of manure-based fertilizers on pathogen contamination; **iii)** testing the effect of biological buffering on pathogen persistence; **iv)** running MRA models and formulating risk mitigation strategies; **v**) confirmation of risk factors identified in WP2.

A- work carried out and results obtained

Experimental plans for the studies to be conducted within WP3 have been coordinated among the PathOrganic partner institutes so that the individual studies are highly complementary. Outlines of the experiments to be performed at the individual labs were developed during the 3rd PathOrganic Consortium Meeting in Frick/ Switzerland. Study design and co-ordination among partners were discussed in detail during the 4th PathOrganic meeting in March 2009; and first experiments were set up soon after.

Ad Task 3i (plant effects)

Experiment: Colonization behavior of *Listeria monocytogenes* and *Salmonella enterica* ssp. Weltevreden in spinach and corn salad (DE)

Experimental set-up: The minimal inoculation dose of *Listeria monocytogenes* sv. 4b and sv. 1/2a EGD-E and *Salmonella enterica* ssp. weltevreden that is needed for the colonization of spinach and corn salad plants was determined. For colonization behaviour and localization analysis the colonizing bacteria sterile plant seedlings were inoculated with GFP (Green fluorescent Protein) tagged strains of *Listeria monocytogenes* sv. and sv. 1/2a EGD-E at a concentration of $4x10^6$ CFU/ml. With this technique in combination with confocal laserscanning microscopy (CLSM) the inoculated bacteria could be detected and localized on the plant surface or endophytically.

Results and Conclusions:
(a) Phytoboxes-experiment: Salmonella enterica ssp. weltevreden showed a higher ability to colonize plant root and shoot than the *Listeria monocytogenes* serotypes. The root compartment was colonized in all plants and at all inoculation doses. In the spinach shoot compartment still two out of three plants were colonized at the lowest inoculation doses where as no colonization was detectable for corn salad shoots at the same inoculation dose, which leads to the suggestion that there is a plant species effect.
Both *Listeria monocytogenes* serovariations showed a similar colonization behavior. Spinach roots of nearly all plants were colonized at the lowest inoculation dose. For corn salad roots in comparison less colonized plants were detected at low inoculation doses. The analysis of the shoot compartment also revealed that spinach was colonized at lower inoculation doses compared to corn salad by both *Listeria monocytogenes* serovariations.
(b) Soil experiment: Salmonella enterica ssp. weltevreden showed a higher ability of colonizing plant root and shoot Than the *Listeria monocytogenes* serovariations used.
Plant roots (were more often found colonized by *Salmonella enterica* ssp. weltevreden compared to shoot. There also was a plant species effect detected with more colonized spinach root and shoot samples compared to corn salad. Furthermore samples fertilized with spiked slurry (were colonized more often compared to spiked manure fertilized samples.

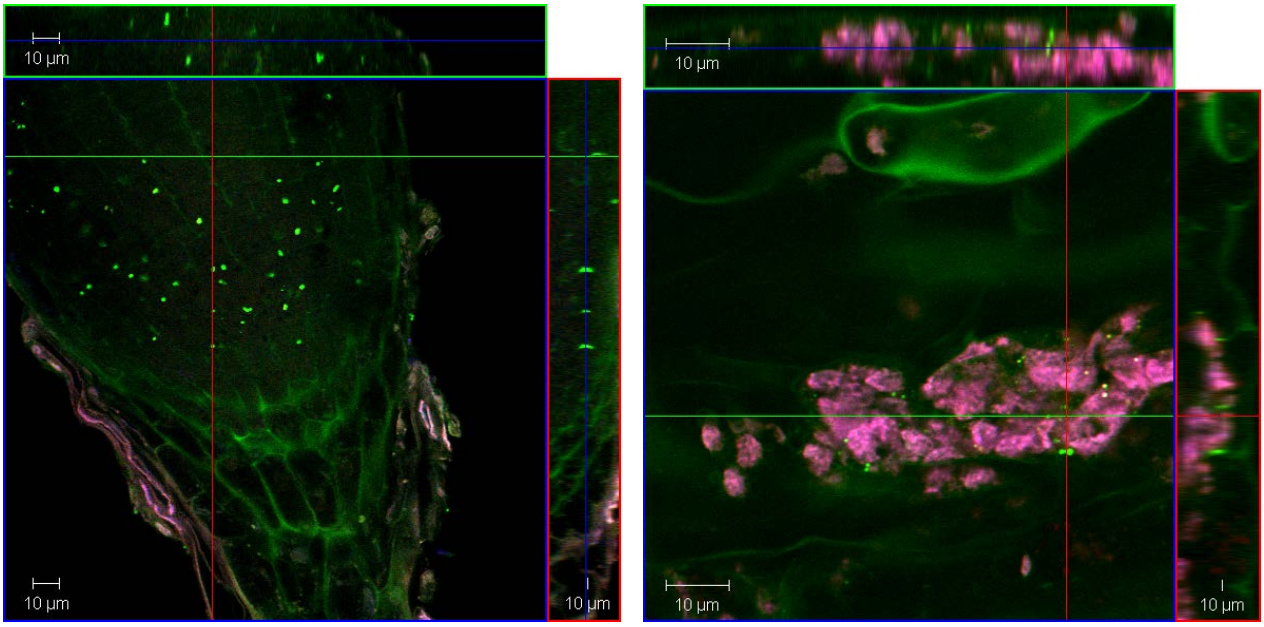

Fig. 7 CLSM images of plants inoculated with GFP tagged *Listeria monocytogenes* serovariations; 1: Root tip a of spinach plant inoculated with *Listeria monocytogenes* sv. 1/2a EGD-E GFP tagged; 2: Leaf of a spinach plant inoculated with *Listeria monocytogenes* sv. 4b GFP tagged;

Experiment: Effects of plant genotype and soil management history on plant colonization by *S. aureus* (AIT + BOKU, Austria)
Experimental set-up: The experiment aimed at assessing how and to what extent *S. aureus* colonizes plants, and whether the plant genotype has an influence on the colonization performance. Effects of soil fertilization and management history were considered by using soils from the MUBIL long-term experimental site. Hence, 4 different soils (DV I green manure, DV II compost, DV III animal manure, DV IV mineral fertilizer) were inoculated with manures previously spiked with *S. aureus*. Successively, soils were planted with spinach (varieties Gamma and Butterfly). After 8 weeks, plants and rhizosphere soil were sampled, and the prevalence and abundance of *S. aureus* in the rhizosphere and on the surface and within the plants were

examined. *S. aureus* was analyzed by plating on Parker-Baird-medium, both following enrichment of spinach leaves and rhizosphere in Giolitti-Cantoni medium and without enrichment.
Results:
•*Staphylococcus aureus* declined rapidly in soil within 8 weeks.
•*Staphylococcus aureus* is able to internally colonize spinach.
•*Staphylococcus aureus* showed no significant differences in colonization behavior or survival in the various fertilization treatments or in the two spinach cultivars.
•*Staphylococcus aureus* generally showed a low persistence in the soil and rhizosphere.

Ad Task 3ii (manure management effects)

Experiment: Persistence and spread of *Salmonella enterica* serovar Weltevreden in soil and on spinach plants (SLU, Sweden)
Experimental set-up: S. Weltevreden was inoculated into cattle slurry at three different concentrations corresponding to 10^4, 10^5 and 10^6 cells g^{-1} soil before addition to soil that was subsequently planted with spinach seeds in plastic pots. In a second experiment, S. Weltevreden was directly inoculated into the soil, 14 days after sowing at a bacterial density of 10^6 cells g^{-1} soil. All the pots were incubated in a phytotron at SLU, Uppsala, Sweden, and were sampled at 0, 7, 14, 21 and 28 days postinoculation. At the sampling occasions, DNA was extracted from soil, roots and shoots, respectively, followed by real-time PCR application (Salmonella-specific primerstargeting a 119-base pair fragment of the *invA* gene) to the different materials.
Results and Conclusions: S. Weltevreden seemed to disperse from manure to spinach roots, and, in some cases, S. Weltevreden present in the phyllosphere had the ability to persist for the entire evaluation period. The results show that S. Weltevreden is capable of persisting in soil, roots and shoots for prolonged periods.

Experiment: Persistence of two *Campylobacter jejuni* strains in soil and on spinach plants (SLU, Sweden)
Experimental set-up: C. jejuni was inoculated into cattle slurry at the three different concentrations before addition to soil that was subsequently planted with spinach seeds in plastic pots. In a second experiment, two *C. jejuni* strains were directly inoculated into the soil, 14 days after sowing at a bacterial density of 10^6 cells g^{-1} soil. All the pots were incubated in a phytotron at SLU, Uppsala, Sweden. The pots were sampled at 0, 7, 14, 21 and 28 days postinoculation. At the sampling occasions, DNA was extracted from soil, roots and shoots, respectively, followed by real-time PCR application (taxa-specific primers targeting the *mapA* gene) to the different materials.
Results and Conclusions: This study confirmed that different strategies for inoculation of *C. jejuni* contribute to the persistence of the bacterium in soil, roots and shoots. Upon inoculation of the bacteria into manure prior to soil application, the amount of *C. jejuni* subsequently recovered in soil was higher than that from treatments involving the addition of *C. jejuni* cells to the soil after plant emergence. Irrespective of the bacterial inoculation dose and strategy employed, the *C. jejuni* content in soil remained relatively constant. There were no major differences between the two *Campylobacter jejuni* strains in *mapA* gene abundance for the different environments and sampling days.

Experiment: Effects of manure treatment on persistence in soil and plant colonization performance of *Staphylococcus aureus* and *Salmonella enterica* (AIT + BOKU, Austria)
Experimental set-up: Various types of differently treated animal manures were amended with *S. aureus* and *S. enterica* bacteria and successively transferred to the soil. Colonization of the soil and plants by the two different pathogens was studied.
Results and Conclusions:
• Both *Salmonella* and *Staphylococcus* declined rapidly in soil within 8 weeks.
• However, after the planting of spinach, *Salmonella* increased again.
• *Staphylococcus* declined more rapidly than *Salmonella*, and cell numbers did not increase after planting.
• Manure type has no significant impact on the survival of the bacteria (one-way ANOVA, unpaired t-test, $p<0.05$).

Experiment: Transport and survival of *Salmonella* Typhimurium bacteriophage, *Escherichia coli* and *Cryptosporidium parvum* through intact sandy clay loam soil cores following surface application and injection of slurry. (UC-LIFE, Denmark; in cooperation with a national funded project entitled "PATHOS")

Experimental set-up: The objective of this study was therefore to evaluate how injection and surface application of pig slurry on intact sandy clay loam soil cores influenced the leaching of *Salmonella* Typhimurium bacteriophage 28B, *E. coli* and *Cryptosporidium parvum* oocysts.

Results and Conclusions: Significantly enhanced leaching of phage 28B and oocysts following injection versus surface application was seen, whereas leaching of the indigenous *E. coli* was not affected by the application method. Preferential flow was the primary transport vehicle and the diameter of the fractures in the intact soil cores facilitated transport of all sizes of microbial tracers under natural weather conditions.

Ad Task 3iii (biological buffering)

Experiment: Persistence of inoculated *Salmonella* Weltevreden in soils from the DOK field experiment (**CH**)

Experimental set-up:

Soils were obtained from DOK long-term field experiment near Basel. In this experiment bio-organic, bio-dynamic, conventional (with and without farm yard manure application) and an unfertilized farming systems have been established in 1978. The persistance of inoculated *Salmonella* Weltevreden was assessed in pot experiments with field soils from the DOK long-term experiment. Five replicate pots of each treatment were filled with 250 g soil and inoculated with $15*10^8$ cfu/ml soil *Salmonella* Weltevreden. Soil samples were taken after 0, 2, 5, 8, 16, 35, 42 and 56 days (experiment A). Rhizosphere and bulk soil of Lamb's lettuce samples were harvested after 35 days growing in inoculated soil (experiment B).

Results and Conclusions:

In experiment A *Salmonella* Weltevreden decreased significantly over time in bulk soil (p=0.001), from $1.66*10^8 \pm 1.54+10^8$ cfu/g soil to $3.03*10^5 \pm 1.89+10^5$ cfu/g soil on average. Although persistence of *Salmonella* Weltevreden tended to be higher in treatments with double the amount of organic fertilizer the differences were not significant ($P>0.05$). Among the treatments no significant difference occurred from day 0 to day 16 ($P>=0.05$). After 35 days significant differences occurred ($P<0.05$). Lowest persistence was found for *Salmonella* Weltevreden in unfertilized soil, where neither organic nor mineral fertilizers have been applied, while highest persistence was observed in organically as well as conventionally managed soils. These results indicated that *Salmonella* Weltevreden persistence may be increased in fertilized soils, regardless of the fertilizer type applied.

In contrast to bulk soil after 35 days of experiment A *Salmonella* Weltevreden showed no significant differences between treatments in rhizosphere and surrounding bulk soil ($P>0.05$) when plants were introduced to the system (experiment B). These results indicate that higher nutrient availability especially through root exudates lowers the differences for persistence of *Salmonella* Weltevreden between treatments.

Experiment: The genome of a European fresh vegetable food safety outbreak strain *Salmonella enterica* subsp. *enterica* serovar Weltevreden and its comparison to other *Salmonella enterica* serovars. (**CH**)

Experimental set-up:

The genome of *Salmonella enterica* subsp. *enterica* serovar Weltevreden strain 2007-60-3289-1 was sequenced. This strain was isolated from alfalfa sprouts and we compared the genomes of this strain isolated from a vegetable with another *S.* Weltevreden strain (SL484), isolated from scallop imported into the USA from Indonesia in 2002. Comparative genomics analysis was conducted as well with complete sequences of other publically-available *S. enterica* subsp. *enterica* serovars. The purpose of this study was to use comparable genome analysis is to identify

genetic similarities and differences for unique virulence attributes and different host ranges.
Results and Conclusions:
Final genome assembly after linkage resulted in 66 contigs with in total 4,922,273 bp. Genome comparison of *S*. Weltevreden of a vegetable isolate with a meet isolate revealed in high synteny. Differences occurred for insertion of phages or mobile elements like transposases and integrases. A novel plasmid pSW82 was identified that contains 93 predicted genes encosind typical plasmid maintainance and stability proteins, transposases and integrases, and a two-gene typeII non-ribosomal peptide sythase/polyketide synthase. Analysis of all available complete *S. enterica* genomes identified differences for presence of type VI secretion systems and carbohydrate pathways. The additional carbohydrate metabolism clusters or the *S*. Weltevreden-specific plasmid might thus enhance survival of this serovar on a broader range of hosts, including plants.

Ad Task 3iv (MRA model and risk mitigation strategies)

The parameters of the conceptual model developed within the project offer potential targets for intervention. If the effect of, for example, an altered practice of manure application or use of alternative plant genotypes is quantified in terms of a decreased probability of pathogen survival in the soil or decreased plant colonization, this can be implemented in the model. However, our current knowledge of the impact of control measures on model parameters (like persistence in the soil or plant uptake of pathogens) is insufficient to directly translate risk mitigation strategies into representative “alternative scenarios” that define a change in model parameter values. Therefore, while providing qualitative information, the model cannot be applied for the quantitative evaluation of risk mitigation strategies as originally planned.
By using a more generic approach towards understanding the mechanisms of food contamination with human pathogens, the model strengthens our insights in the essential factors behind plant contamination by human pathogenic bacteria. Furthermore, it identifies additional data that are required for a more advanced model.

A MRA model needs valid input data for every step considered to influence the persistence and/or proliferation of human pathogenic bacteria in a certain chain – in this case the production chain from planting of lettuce to harvest. The current model identified steps where a decline of bacteria is likely to occur; in the manure during storage and in the manure-amended soil before/after planting, and although variations in physical and chemical conditions appear to affect this die-off, some rough estimates can be found from literature and experiments.
However, the possible interactions between the plant and bacteria are much more complex and ‘unpredictable’. The performance of quantitative risk assessments requires sufficient and adequate data for every single step pointed out to be of importance in the model pathway. Typically, several unexpected data gaps are identified during the development of the risk assessment, and the project should have the flexibility to fill up some of those data gaps, either by additional research or by expert elicitation. Accordingly, a strong collaboration between risk assessors and ‘experimental workers’ is required from an early start in the planning and performance of risk assessments. This is important in order to ensure generation of data that can be readily implemented into the model and cover the identified gaps in knowledge and existing data.

Three experiments were set up by UC-LIFE, Denmark, to obtain information on fecal contamination of soil and produce (tomatoes and potatoes) as well as pathogen transport in soil water following application of pathogens/fecal bacterial indicators. Data were generated in collaboration with the SAFIR project (FP6) for the use in MRA in PathOrganic.

Experiment1: Fecal contamination and hygiene aspect associated with the use of treated wastewater and canal water for irrigation of potatoes (*Solanum tuberosum*).
Experiment2: Fecal contamination and health aspects of soil and tomatoes (*Solanum Lycopersicum*) irrigated with partially treated wastewater.
Experiment3: Leaching of human pathogens in repacked soil lysimeters and contamination of

potato tubers under subsurface drip irrigation.

Main Results and Conclusions:

- No relationship was found between *E. coli* concentrations in irrigation water, soil and produce. This indicates that subsurface application of organic wastes like low quality water and slurry can be practiced while ensuring food safety and protecting the health of consumers and farmers.
- Elevated levels of *E. coli* were found in irrigation water but low concentrations in soil and even lower concentrations on tomatoes. This highlights the importance of the external environment as a source of fecal contamination which needs further investigation.
- Findings of bacterial pathogens and phage 28 on potato samples suggest that the main risk associated with subsurface application of liquid/solid wastes containing high concentrations of pathogens, e.g. here drip irrigation with low quality water, is fecal contamination of root crops, in particular those consumed raw.

Ad Task 3v (risk factors)

The conceptual model together with the experimental essays pointed out parameters that can be considered targets for measuring the efficiency of risk interventions. Major conclusions from the WP3 experiments and the model work are:

- Following manure application to the soil, there will be a decline in the numbers of pathogens, but the specific decline rate depends on physical and chemical conditions.
- The initial pathogen load as well as the time of manure application are important factors for the risk of crops being colonized by pathogens.
- The interactions between crops and pathogens present in manure-amended soil and the fate of pathogens in/on crops during the growth period are much harder to estimate under field conditions than in greenhouse experiments. A key factor here is whether the pathogens experience conditions which allow that they may proliferate.

B- comments on deviations from the original plan

In WP3, important factors were identified that influence the transfer of bacterial pathogens to crops via manure amended soil. Nevertheless, it was realized that such data are not readily applicable in MRA models. There were more knowledge gaps than anticipated, which hindered completing a model that translates research data into mitigation strategies regarding human health risks (which should be the final output of the MRA model). Thus, we chose a more generic approach towards understanding the mechanisms of food contamination with human pathogens and built a model that strengthens our insights in the essential factors behind plant contamination.

WP 4	**Final Risk Assessment, Communication and Recommendations**

Responsible partner: 3, FiBL, Gabriela Wyss/ Martin Koller

Description of work:

The overall aim of WP4 is to summarize all findings referring to critical control points and potential risks in organic vegetable production (WP 2 and 3) and to elaborate specific recommendations. A model for risk assessment of human pathogen spread and persistence is adjusted in collaboration with project participants and stakeholders in order to ensure the relevance of the project for organic plant production.

A final workshop (within the last six months) with organic producers, European organic producer organizations, marketing chain representatives and politicians will be held to discuss the critical control points and recommendations and to check the suitability of the proposed risk model. Contacts with stakeholders will also ensure that the results of the research will be communicated to the real world.

WP4 encompasses the following **tasks**:

i) development of a model based on "@Risk" for contamination of lettuce with enteric pathogens serving for the final microbiological risk assessment;
ii) summarizing all the risks identified based on quantitative (model) and qualitative (questionnaire) assumptions as well as on experimental findings;
iii) communication of results to end-users, the EU and national governments.

Final report on work carried out and results compared to the original plan/WP aims:

A- work carried out and results obtained

Ad Task 4i (model development)

As explained in task 3, a conceptual model was derived from the *E. coli* O157 model by Franz et al (2008). Quantitative *E. coli* data from three fields in Denmark, where we have manure data, field data and plant data, were used to model survival of this faecal indicator and its contamination of lettuce in @Risk using the model of Franz et al. (2008) and some other basic QMRA models by Nauta et al (2008). This model approach explores two routes of crop contamination: via the soil and via splashes. The modeling revealed that the data from one of the fields could not be explained by contamination via one of these routes: the concentrations of *E. coli* on the lettuce were too high for that. For the other two fields, "infectivity" had to be quite high to explain the levels of *E. coli* contamination found. It was also attempted to apply this model for irrigation water, but the available data (UC-LIFE) did not allow this. The current model can be used as a tool to discuss how crops are actually contaminated in the field, and which risk factors are actually thought to be important in the process. So it can be used to check our understanding, build hypotheses, and discuss risk mitigations. However, it is not a risk management tool, simply because we do not have enough (quantitative microbiological) knowledge of the process. This model was presented and discussed at the stakeholder workshop in 2010.
Finally we developed another conceptual model that quantifies the inactivation and proliferation of pathogens in the pathway manure – soil – crop. Experts within the project were asked to provide input, based on the knowledge obtained in the PathOrganic project, specifically data on persistence in the soil and the concentrations of specific pathogens needed to get infection/contamination of the crops. This model was discussed at the meeting in Vienna 2011 and it is still in development. Data on inactivation and critical concentrations in the soil for plant infection provided by PathOrganic will be applied to this model, but there is still a bottleneck for getting data on the manure storage times, fertilizing practices and realistic initial concentrations of pathogens that reflect current practices for different crops / pathogens/ soil types. If we can get access to this information, this model will result in a tool that is useful to support discussions on the health risks of the application of manure as fertilizer of crops. We aim to publish the present model work in a peer-reviewed paper, possibly in combination with a similar model for irrigation water.

Ad Task ii (summarizing risks)

Recommendations for improved farm practice were developed based on literature findings and research outcomes of PathOrganic. Before used as inputs for regulations, these and other recommendations should be evaluated in field experiments. (This was not in the scope of the present project.)

- Feeding roughage and not concentrates to cattle minimizes the propagation of pathogenic *E. coli* (including EHEC strains). This conclusion is based on the following literature findings:

Diez-Gonzalez F. et al. 1998. Grain feeding and the dissemination of acid-Resistant Escherichia coli from cattle. Science 281, 1666-1668
Callaway TR, Elder RO, Keen JE, Anderson RC, and Nisbet DJ (2003) Forage Feeding to Reduce Preharvest Escherichia coli Populations in Cattle, a Review. J. Dairy Sci. 86:852–860

- Manure should be composted whenever possible.
- If not (aerobically) composted, storage of manure and slurry for 4 months without adding new material is recommended.
- Shallow incorporation, instead of deep ploughing or surface application. Surface application would allow a faster decrease of pathogens, but leads to higher ammonia evaporation.
- Solid manure is preferable to slurry.
- After planting and sowing, manure and slurry should not be applied and is even prohibited under certain organic regulations and Global GAP.
- In vegetable crops with a short cropping period (< 100 days), which are intended for raw consumption (e.g., lettuce) and for raw convenience food (e.g., chopped salad), fresh manure/slurry should be applied at the latest 4 months before crop establishment.

During the model work it became evident that is was not feasible to make a risk assessment as strong as anticipated and consequently it was difficult to provide specific risk estimates. The model can nonetheless provide insight in the basic dynamics of plant contamination, and the available data illustrate that this is poorly understood. This provides a basis for discussion on the required data collection, further development of the model, and control options.

Ad Task 4iii (communication)

A workshop with 25 participants was held at FiBL in Frick at 16th April 2010.

A leaflet was elaborated to communicate risk factors and recommendations concerning the use of manure in vegetable production. The leaflet is available in English at www.shop.fibl.org order number 1562. Translations in German and probably other languages will follow.

Besides presenting the project results, new research questions arising from the project were formulated and challenges for the future were addressed during the final workshop.
New research questions:

- Plant breeding: Can we provide vegetable cultivars that are less prone to being colonized by human pathogens?
- Are there bacterial strains (possibly also plant growth-promoting) which can out-compete „invading“ pathogens?
- A focus on post-harvest practices is needed for studying how to prevent further proliferation of human pathogens.

Challenges in the future regarding bacterial pathogens in/on vegetables:

- Increasing demand for ready-to-eat vegetables and potential consequences for product safety
- Increasing global trade, also of organic products, and potential consequences for product safety
- Climate change and increased plant colonization by human pathogens

B- comments on deviations from the original plan

A valid quantitative microbiological risk assessment (QMRA) strongly depends on sufficient and adequate quantitative data and on knowledge of the impact of processes along the farm to fork pathway. The PathOrganic project yielded valuable information, but not enough to build the models that were anticipated at the start of the project. The lack of data led to a necessary change in model strategy, as stated above.
The potential fecal contamination of lettuce via manure amended soil was addressed via the use of

quantitative data of *E. coli* as a fecal indicator in manure and the harvested lettuce. These data were used in a conceptual model that pointed to parameters, which can be considered targets for measuring the efficiency of risk interventions. However, our knowledge is insufficient to translate practical risk mitigation strategies into alternative scenarios for risk assessment.
Next, we developed a conceptual model that quantifies the inactivation and proliferation of pathogens in the pathway manure – soil – crop. Experts within the project provided input, based on the knowledge obtained in the PathOrganic project, specifically data on persistence in the soil and the concentrations needed to get infection/contamination of the crops. This model will be submitted for publication in a peer reviewed paper.

4. Publications and dissemination activities

4.1 List

Note: the report should contain all the publications and dissemination activities of the project. Publications should have been loaded in Organic Eprints, but some dissemination activities might not be in Organic Eprints and still have to be listed below. Part of the list required below can be extracted and pasted by doing a search in Organic Eprints, and others added manually. The resulting list should be clear and complete, whether the tables below are used or a search in Organic Eprints.

Project website(s)

Address		**Authors:** (name + institution acronym)	**When was it last updated**	**Language**	**Comments**
	http://pathorganic.coreportal.org/	Hackl, Fenzl, Sessitsch AIT	June 2010	English	The website has been removed; contents have been saved

Deliverables

Planned/ actual date	**Title:**	**Authors:** (name + institution acronym)	**Where is it available**	**Language**	**Comments**
23-24 Jan 2008	Lab-workshop among project participants on method harmonization	Schmid, Hartman, Hofman HGMU	n.a.	English	
January 2008	Protocols on methods for the detection of pathogens in complex biological matrices	PathOrganic consortium	Deliverables book	English	
Sept 2008	Final protocols for sampling and surveying developed	PathOrganic consortium	Deliverables book	English	
April 2010	Conceptual model on pathogen transfer developed	DTU-Food, CU-LIFE	Publication planned	English	
Feb 2008	Information to farmers (leaflets)	Koller FiBL , Hackl, Sessitsch AIT + PathOrganic consortium	Deliverables book	English	
15.7.2011	Manure for Vegetables. Farm practice recommendations for minimizing human pathogenic bacteria contamination in vegetable production. Technical guide «Manure Use in for Vegetables» Order number 1562 International edition © FiBL 2011	Martin Koller, FiBL + PathOrganic consortium	www.shop.fibl.org	English	
July 2011	Identification of Critical Control Points for organic vegetable crops. Report by FiBL, July 2011 Available with "restricted access" at http://orgprints.org/20389/ (13.Jan.2012)	Bettina Landau, Martin Koller, Paul Mäder	FiBL, deliverables book (executive summary)	English	

Reviewed papers (with full reference)

Planned / actual date	Title:	Authors: (name + institution acronym)	Name of Magazine, volume, pp. etc.	Language	Comments
2010	Faecal contamination and hygiene aspect associated with the use of treated wastewater and canal water for irrigation of potatoes (*Solanum tuberosum*).	Forslund et al. UC-LIFE	Agricultural Water Management, 98: 440–450	English	
2011	Leaching of human pathogens in repacked soil lysimeters and contamination of potatoes under subsurface drip irrigation	Forslund et al. UC-LIFE	Water Research (in press)	English	
2011	Transport and survival of *Salmonella* Typhimurium bacteriophage, *Escherichia coli* and *Cryptosporidium parvum* through intact sandy clay loam soil cores following surface application and injection of slurry	Forslund et al. UC-LIFE	Applied and Environmental Microbiology (submitted and in review)	English	
2011	Fecal contamination and health aspects of soil and tomatoes (*Solanum Lycopersicum*) irrigated with partially treated wastewater	Forslund et al. UC-LIFE	(In prep)	English	
2011	Faecal contamination of lettuce after manure application	Jensen et al, DTU-Food/CU-LIFE	International Journal of Food Microbiology (in prep.)	English	
2011	The importance of manure and irrigation water as sources of contamination of fresh produce: a modelling approach	Nauta et al DTU-Food/CU-LIFE	In prep	English	
2011	Persistence and spread of Salmonella enteric serovar Weltevreden in soil and on spinach plants	Arthurson, V. (SLU); Sessitsch, A. (AIT); and Jäderlund, L. (SLU).	FEMS Microbiology Letters 314 (2011), 67-74.	English	
2011	Persistence of two Campylobacter jejuni strains in soil and on spinach plants	Jäderlund, L. (SLU); Sessitsch, A. (AIT); and Arthurson, V. (SLU).	Applied and Environmental Soil Science vol 2011, 1-7.	English	
2011	Genome of a European Fresh-Vegetable Food Safety Outbreak Strain of *Salmonella enterica* subsp. *enterica* Serovar Weltevreden	Brankatschk[1], K[1]., Blom[2], J., Goesmann[2], A., Smits[1], T.H.M., Duffy[1] B. [1]ACW; [2]CeBiTec, University of Bielefeld	J Bac Apr. 2011; p. 2066	English	
2011 (submitted)	Dispersal of *Salmonella* by rain splash onto tomato plants	Cevallos-Cevallos, J.M., Danyluk, M.D., Gu, G., Vallad, G.E., and van Bruggen, A.H.C.	Journal of Food Protection (submitted)	English	
2011 (in press)	Internal colonization of *Salmonella enterica* serovar Typhimurium in tomato plants.	Gu, G., Hu, J., Cevallos-Cevallos, K.M., Richardson, S.M., Bartz, J.A.	PlosONE (in press).	English	

		and van Bruggen, A.H.C.			
2011	Influence of aerobic and anaerobic conditions on survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in Luria-Bertani broth, farm-yard manure and slurry	Semenov, A.V., van Overbeek, L., Termorshuizen, A.J., and van Bruggen, A.H.C.	J. Env. Management 92: 780-787	English	
2011	Transfer of enteric pathogens to successive habitats as part of microbial cycles	Semenov, A.M., Kupriyanov, A.A. and van Bruggen, A.H.C.	Microb. Ecol. 60: 239-249	English	
2011	COLIWAVE: a simulation model for survival of *E. coli* O157:H7 in dairy manure and manure-amended soil	Semenov, A.V., Franz, E., and van Bruggen, A.H.C.	Ecological Modelling 221: 599-609	English	
Planned 2011	Comparative Genomic Analysis of *Salmonella enterica* subsp. *enterica* serovar Weltevreden foodborne strains with other serovars.	Brankatschk[1], K[1]., Blom[2], J., Goesmann[2], A., Smits[1], T.H.M., Duffy[1] B. [1]ACW; [2]CeBiTec, University of Bielefeld	Intl J Food Microbiol	English	In preparation
Planned 2011	Prevalence of Human Pathogenic Bacteria in Manure Amendments Applied in European Conventional and Organic Fresh Vegetable Production Systems.	Jäderlund, L., Brankatschk, K., Arthurson, V. + all	Appl Environ Microbiol	English	In preparation
Planned 2011	Incidence of human pathogenic bacteria in field vegetables fertilized with animal manures	C. Fenzl, E. Hackl, K. Brankatschk, L. Jäderlund, A. Hofmann, V. Arthurson, A. N. Jensen, G. Wyss, T. Rinnofner, M. Koller, J.K. Friedel, B. Duffy, M. Schmid, A. Hartmann and A. Sessitsch	FEMS Microbiology Letters	English	In preparation

Presentations/papers at scientific conferences (oral presentations, papers, leaflets, posters, etc.)

Planned / actual date	**Type and Title of contribution:** (also mention if a partner was keynote speaker)	**Conference:**	**Partners involved:** (partner acronyms)	**Type of audience** (General public, higher education, researchers, industry, farm sector, advisors etc.)	**Size of audience**	**Countries addressed**
8-10 Oct 2008	The PathOrganic project – evaluating and reducing risks regarding human pathogens in organic fresh produce (conference paper)	MoniQA International Conference "Increasing Trust in Rapid Analysis for Food Quality and Safety"	all	Researchers, industry, policy makers	About 150	international
11-13 Feb 2009	PathOrganic – risks and recommendations regarding human pathogens in organic vegetable production chains (conference paper)	10. Wissenschaftstagung Ökologischer Landbau	all	Researchers, higher education, farm sector, advisors	About 500	international
28 June- 2	Transport and survival of *Salmonella*	FEMS 2009 - 3rd	UC-LIFE	Researchers	About 700	International

July 2009	Typhimurium bacteriophage 28B and *Cryptosporidium parvum* from slurry applied to intact clay soil cores (abstract and poster)	Congress of European Microbiologists, Gothenburg, Sweden				
28 June- 2 July 2009	Surveying Pathogenic Bacteria in Vegetables (abstract and poster)	FEMS 2009 - 3rd Congress of European Microbiologists, Gothenburg, Sweden	AIT + consortium	Researchers	About 700	International
28 June – 2 July, 2009	Incidence of thermophilic Campylobacter spp. in animal manure and organically produced vegetables.	FEMS 2009 - 3rd Congress of European Microbiologists, Gothenburg, Sweden	all	Researchers, industry.	About 700	international
31 Aug – 2 Sept 2010.	Faecal contamination of lettuce with after manure application (abstract and poster)	Food Micro 2010, Copenhagen, Denmark	DTU Food/UC-LIFE	Researchers	About 300	international
31 Aug – 2 Sept 2010.	Faecal contamination of soil and tomatoes irrigated with partially treated wastewater and associated health risks (poster)	Food Micro 2010, Copenhagen, Denmark	UC-LIFE	Researchers	About 500	international
18-20 May,	Contamination of lettuce with antibiotic resistant *E. coli* after slurry application. (Oral presentation)	First Int. Conference on Organic Food Quality and Health Research, Prague, Czech Republic	DTU Food/UC-LIFE	Researchers	150	International
15-19 June, 2009	Incidence of thermophilic *Campylobacter* spp. in animal manure and organically produced vegetables.	10th Symposium on Bacterial Genetics and Ecology (BAGECO), Coexisting on a changing planet.	all	Researchers, industry,	About 250	international
31.10.2008	Poster: Microbial food safety in fresh produce production chains	ASPSA2008 "Annual Symposium of the PhD Program in Sustainable Agriculture"	ACW, ART, FIBL, ETH, AIT	General public but mostly PhD students from ART	About 30	Switzerland
27.08.2009	Poster: Microbial food safety in fresh vegetable production chains	ASPSA2009	ACW, ART	General public but mostly PhD students from ART	About 30	Switzerland
24-26.03.2010	Poster: Genome of a *Salmonella enterica* serovar Weltevreden plant-	SWIMM2010 "The 5th Swiss Molecular	ACW	Scientists, mostly PhD students	About 60	Switzerland

	associated outbreak strain from Scandinavia	Microbiology Meeting"				
24-25.05.2010	Poster: Genome of a *Salmonella enterica* serovar Weltevreden plant-associated outbreak strain from Scandinavia	Annual Meeting of Swiss Society for Microbiology	ACW	Researchers	About 300	International
7-11.06.2010	Poster: Complete genome sequence of a plant-associated Salmoella enterica serovar Weltevreden strain from a sprout outbreak in Scandinavia	International Conference on Plant Pathogenic Bacteria 2010 (ICPPB)	ACW	Researchers	About 150	International
30.08-03.09.2010	1. Poster: Microbial Food Safety In Fresh Vegetable Production Chains 2. Poster: Genome of a *Salmonella enterica* serovar Weltevreden plant-associated outbreak strain from Scandinavia	FoodMicro2010: 22nd International ICFMH Symposium	all	Researchers, Representatives, Public health officals		International
25.11.2010	Presentation: Persistence of inoculated *Salmonella* Weltevreden in soils from the DOK field experiment	ASPSA2010	ACW, ART, FiBL	General public but mostly PhD students from ART	About 30	Switzerland
6-10.08.2011	Poster: Comparative genomics of *Salmonella enterica* serovar Weltevreden plant and seafood isolates	**American Phytopathological Society**	ACW	Researchers	?	International

Presentations/papers at other conferences and meetings (oral presentations, papers, leaflets, posters etc.)

Planned / actual date	**Type and Title of contribution:** (also mention if a partner was keynote speaker)	**Conference/title:**	**Partners involved:** (partner acronyms)	**Type of audience** (General public, higher education, researchers, industry, farm sector, advisors etc.)	**Size of audience**	**Countries addressed**
31 May 2011	Ways to detect pathogens	Working group Molecular Diagnostics for infectious diseases	PRI	Researchers, Industry	40	Netherlands

Book chapters

Planned / actual date	**Type and Title of contribution:**	**Book title**	**Partners involved:** (partner acronyms)	**Type of audience** (General public, higher education, researchers, industry, farm sector, advisors etc.)	**Size of audience**	**Countries addressed**

Popular articles and other dissemination activities (press releases, interviews etc.)

Planned / actual date	**Title of contribution:**	**Type of contribution** (presentation, leaflet, poster etc.)	**Partners involved:** (partner acronyms)	**Type of audience** (General public, industry, farm sector, advisors, policy makers, public authorities, higher education, researchers, etc.)	**Language**	**Countries addressed**
9.3.-11.3.08	Risks and recommendations regarding human pathogens in organic vegetable production chains	Poster	HMGU	researchers	English	Germany
March 2008	Forschungsprojekt PathOrganic. Risiken und Empfehlungen betreffend das Vorkommen von Humanpathogenen im biologischen Gemüseanbau	Leaflet	AIT, BOKU	Information to farmers	German	Austria
April 2008	Forskningsprojektet "PathOrganic" [The research project "PathOrganic"]	Leaflet	DTU-Food	Information to farmers	Danish	Denmark
June 2008	Den Bakterien im Gemüse auf der Spur	Article	AIT	Researchers, general public	German	Austria
09.09.2008	(Forschungstreffen BAG-BVET-BLW "Sicherheit entlang der Lebensmittelkette")	poster	ACW	General public, research groups working on food safety	English	Switzerland
September 2008	Risks and Recommendations Regarding Human Pathogens in Organic Vegetable Production Chains (PATHORGANIC)	Poster	AIT	Researchers	English	Austria
8.10-10.10.08	The PathOrganic project – evaluating and reducing risks regarding human pathogens in organic fresh produce	Poster	all	Researchers, industry, policy makers	English	international
31.10.2008	(4th Annual Symposium of the PhD Program in Sustainable Agriculture)	poster	ACW, ART	Gerneral public but mostly PhD students from ART	English	Switzerland
October 2008	Gefährliches Bio-Gemüse	Press release	AIT	General public	German	Austria
November 2007	Infectious matter in organic fruit and vegetables	Press release, at institute homepage	DTU-Food, CU-LIFE	General public	Danish	Denmark
November 2007	Forebyggelse af smitstoffer I økologisk frugt og grønt -[Prevention of infectious matter in organic fruit and vegetables]	News Letter (FØJOenyt)	DTU-Food, CU-LIFE	Organic organisations, general public	Danish	Denmark
November 2008	Den Bakterien im Gemüse auf der Spur	Article	AIT	Researchers, general public	German	Austria
11-13 Feb 2009	PathOrganic – Risks and Recommendations Regarding Human Pathogens in Organic Vegetable Production Chains	Poster	all	Researchers, higher education, farm sector, advisors	English	international

23. 7. 2011	Hintergründe zu EHEC, Salmonellen und Co. in Gülle, Mist und Boden, Resultate des Forschungsprojektes PathOrganic	Article	FiBL, ACW, ART, AIT, BOKU, HMGU	Organic vegetable growers	German	Germany, Austria, Switzerland
6.8.2011	Hofdüngereinsatz im Gemüsebau	Article	FIBL, ACW, ART	Farmers (conventional and organic)	German	Switzerland
16.8.2011	Hofdünger im Gemüsebau mit Vorsicht einsetzen	Article	FIBL, ACW, ART	Farmers (conventional and organic)	German	Switzerland
27.10.2011	Pathogene Mikroorganismen in Mist: Resultate von PathOrganic	Presentation	FiBL	Organic vegetables advisors	German	Germany, Austria, Switzerland
17.11.2011	Hofdünger im Biogemüsebau sicher einsetzen: Was gilt es zu beachten? Wie in der Praxis umsetzen?	Presentation	FiBL	Organic vegetables growers	German	Switzerland

Internal reports and proceedings, newsletters, web communication and other dissemination activities etc.

Planned / actual date	**(No.) and title**	**Type:** Deliverable, proceedings, internal report, newsletter, web communication	**Partners involved:** (partner acronyms)	**Type of users addressed** (General public, higher education, researchers, industry, farm sector, advisors etc.)	**Language**	**Countries addressed**
15.03.08	Risks and recommendations regarding human pathogens in organic vegetables	Internal report	HMGU	HMGU staff department	Germany	
2011	[In English - Contamination risk associated with application of animal manure]	newsletter	DTU Food/CU-LIFE	Farm sector, advisors	Danish	DK
27.4.2011	Factsheet humane bacteriën vanuit de plant	Fact sheet	PRI	Researchers, industry, policy makers	Dutch	The Netherlands

4.2 Further possible actions for dissemination

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

Bettina Landau, Martin Koller, Paul Mäder. PathOrganic - Identification of Critical Control Points for organic vegetable crops. Report by FiBL, July 2011

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

Manure for Vegetables. Farm practice recommendations for minimizing human pathogenic bacteria contamination in vegetable production. Technical guide «Manure Use in for Vegetables» Order number 1562 International edition © FiBL 2011; available at www.shop.fibl.org

The leaflet is based on the major output of the PathOrganic project and could usefully be translated in Danish, German, Swedish, eventually also French.

4.3 Specific questions regarding dissemination and publications

- Is the project website up-to-date?

The project website has been removed already in 2010. However, it was in general kept up-to-date, and was especially useful for announcing and documenting the stakeholder workshop.

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

Farmers: Leaflets with information for farmers on the project were prepared in all countries where surveys were carried out, and were distributed among the farmers when samples were taken from the individual farms.
Agricultural associations: Farmers and representatives of agricultural associations together with representatives of the national and CoreOrganic funding bodies were invited to participate in the stakeholder workshop.
General public: Non-scientific publications were prepared in several countries
Scientific community: Dissemination of project results was done through presentations at international conferences and via publication in international journals

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

See above. The target groups were reached via the activities given above. However, these activities were set at the end of the project. Therefore, any consequences (such as raised awareness, attention to farm management procedures) are not yet known and need to be specifically investigated.

ANNEX: Protocols

Manure surveys: Sample preparation and enrichment cultures

25 g of manure/ slurry-sample were transferred into 225 ml of sterile medium appropriate for the detection of the specific pathogen (Buffered *Listeria* Enrichment Broth (B*L*EB) for *Listeria sp*., BSEB for *Campylobacter* spp., buffered peptone water for *E.coli/Staphylococcus* sp./*Salmonella sp*.) and homogenized if required. Enrichments for *E.coli/Staphylococcus* sp./*Salmonella* sp. were incubated at 37 °C for 18 hours without aeration, those for *Listeria* sp. at 30 °C for 48 h (addition of selective supplement after 3 h), and those for *Campylobacter* spp. at 37 °C for 4-6 h, followed by 44 h at 41.5 °C.

Microbial DNA-Isolation from manure samples

Molecular analyses were performed on microbial DNA isolated from enrichment cultures (see above) using the Bio101 fast DNA spin kit for soil (MP Biomedicals). Microbial cells were harvested from 2 ml enrichment culture by centrifugation at 5000 rpm for 5 min. The cells were washed two times with 1 x PBS, suspended in 50 µl enzyme solution (containing 300 U ml^{-1} mutanolysine and 500 U ml^{-1} lysostaphin in ultra pure H_2O) and incubated at 37 °C for 30 min. The further procedure followed the manufacturer's protocol except that 978 µl Sodium Phosphate buffer and 122 µl MT buffer were added and the DNA was eluted in 100 µl DES. Quality and quantity of the isolated DNA were analyzed spectrophotometrically and by using horizontal agarose gel electrophoresis following standard procedures.

Chemical analyses of manures and slurries

Analytical procedures for the measurement of chemical properties of manures and slurries varied to some extent between individual laboratories depending on the available analytical equipment. The following procedures were used for Austrian samples:
For the determination of dry matter the samples were dried at 60 °C followed by drying at 105 °C until constant weight was reached. The pH measurements were done on suspensions of 50 g of organic fertilizer in 500 ml $CaCl_2$ (0.01 mol/l) after shaking for 1 h.
Ammonium and nitrate in organic fertilizers (slurry, manure, compost) were determined by using a modification of the method for assessing inorganic nitrogen in soil samples (ÖNORM L1091, 1999). Ammonium and nitrate were measured in extracts that were prepared from 50 g organic fertilizer in 500 ml 0.01 M $CaCl_2$ solution by shaking for one hour and filtration. Nitrate-N was measured according to Navone (1964) using an UV–VIS-Photometer at 210 nm, regarding blank values with the background of humic substances only. Ammonium-N in the extracts was determined by a modified Berthelot reaction using salicylate and dichlorisocyanurate (Krom 1980). The ammonium complex formed was measured photometrically at 675 nm.
For total N measurements the samples were burnt in a LECO C-N analyzer and N2 evolved was determined by gas chromatography. Total P measurements were done using a modified method by Bowman (1989), involving treatment with concentrated sulphuric acid and subsequent determination by spectrocolorimetry of phosphovanadomolybdate (yellow)-molybdene blue as described in Murphy and Riley (1962).

Protocols for pathogen analyses in manures and vegetables

Analysis for *Salmonella* sp. and enterohaemorrhagic *E. coli* O157 by applying a plating method

Analysis of organic fertilizers for *Salmonella* spp. was done according to ISO 6579 Annex D. 225ml buffered peptone water was inoculated with 25 g of manure resp. 25 ml of slurry sample following anaerobic incubation at 37 °C for 18 h for non-selective pre-enrichment. Selective Modified-Semisolid-Rappaport-Vassiliadis media with 10 mg/l Novobiocin was inoculated with 0.1 ml of the non-selective pre-enrichment culture and incubated for 24 h at 42°C. Cultures that show opaque

zones were transferred on selective Xylose-Lysin-Desoxycholat-agar. After 24 h at 37 °C Salmonella positive cultures turn red due to fermentation of xylose and decarboxylation of lysine and show a black center due to hydrogen sulphide production. As a second selective agar Triple Sugar Iron Agar was used. Positive colonies showing again a black center due to production of hydrogen sulphide. Red colonies showing black centers were considered to be positive for *Salmonella* spp.
Analysis of manure and slurry samples for enterohaemorrhagic *E. coli* O157 followed modified protocol of ISO 16654. Sample was selectively enriched by adding 25 g manure or 25 ml slurry to 225 ml modified EC broth containing 0.02 g/l Novobiocin and was incubated for 18 h at 37 °C. For selective Immunomagnetic Separation (IMS) of E. coli O157 Dynabeads anti-E.coli O157 (Invitrogen, Dynal AS, Oslo, Norway) were used. After IMS resuspended beads were transferred onto CT-SMAC and incubated at 37 °C for 24 h. Colorless colonies were considered to be positive for *E. coli* O157.

Analysis for staphylococci by applying a plating method
Analysis of organic fertilizers for staphylococci was done according to ISO 6888-3 with slight modifications. 9 ml of Giolitti and Cantoni broth containing potassium tellurite was inoculated with 1 ml of initial suspension of enrichment culture (see above) following anaerobic incubation at 37 °C for 24 h and 48 h, respectively. Presence of presumptive coagulase-positive staphylococci was indicated by a black color of the medium due to the reduction of potassium tellurite to tellurium. After 24 h, 100 µl medium from presumptive positive tubes were used for inoculating solid Baird-Parker medium in petri dishes, and after 48 h all the remaining tubes were used for inoculation. Plates were incubated at 37 °C for 48 h. After 24 h and after 48 h the plates were visually inspected for the presence of presumptive coagulase-positive staphylococci as indicated by an egg yolk reaction, showing an opaque zone surrounding the colonies. Samples were considered as positive for staphylococci if both reduction of potassium tellurite occurred and the egg yolk reaction was positive.

Analysis for *Listeria* sp. by applying a plating method
Analysis of organic fertilizers for *Listeria* sp. was done according to ISO 1290-1 with modifications. First enrichment step of 25 g sample was conducted in 250 ml Buffered *Listeria* Enrichment Broth with selective supplement and incubated at 30°C under aeration. After 1, 2 and 7 days 10 ml Half Fraser Bullion with selective supplement was inoculated with 100 µl of the enrichment culture and incubated at 37°C under aeration. After 1 and 2 days of incubation in Half Fraser Bouillon 20 µl of the enrichment were plated on OXFORD *Listeria* selective plates (30°C) and PALCAM *Listeria* selective plates (37°C) and incubated for two days at the respective temperature. Samples were considered as positive for *Listeria* sp. if black colored colonies occurred on the selective media plates.
An additional identification of *Listeria monocytogenes* was performed using RAPID´*L.mono* chromogenic media plates (BioRad, Munich, Germany). Therefore 20 µl of the Half Fraser Bouillon subculture were plated and then incubated at 37°C for 48 h. Formation of blue colonies without yellow halo indicate presence of *Listeria monocytogenes*.

Analysis for *Campylobacter* spp. by applying a plating method
Analysis of manure samples with respect to *Campylobacter* species was performed according to ISO 10272-1.

PCR-based analysis of enterohaemorrhagic *E. coli* O157:H7 (EHEC) and virulence genes in manures, slurries and vegetables
For PCR based analysis of enterohaemorrhagic E. coli (EHEC) and virulence genes 2 separate PCRs were carried out. DNA sequences of used primers and sizes of PCR products are shown in Table 1.
First a PCR specific for rfbE-genes of E. coli O157 was used. A 20 µl PCR reaction contained 1 x master mix of HotStarTaq Master Mix Kit (Quiagen, Basel, Switzerland), 0.5 pmol of each primer and 1 µl of DNA template. Thermal cycling conditions were: an initial denaturation at 95 °C for 15 minutes followed by 35 cycles of 94 °C for 30 sec, 66 °C for 30 sec, 72 °C for 30 sec, and a final extension at 72 °C for 10 min (Desmarchelier et al. 1998).

Second for detecting virulence genes Shiga toxin 1 & 2 (*stx1* & *stx2*) and eae encoding intimin-adherence protein (*eae*) a multiplex PCR was carried out. A 20 µl PCR reaction contained 1 x master mix of Multiplex PCR Master Mix Kit (Quiagen, Basel, Switzerland), 1 pmol of each primer and 1 µl of DNA template. Cycling conditions for multiplex PCR were: an initial denaturation at 95°C for 5 min, followed by 35 cycles, each consisting of 90 s at 94°C, 90 s at 64°C, and 90 s at 72°C.

Table 1: Primers used in PCR reactions for detection of enterohaemorrhagic E.coli genes

Gene	Primer sequence, 5'-3'	Size of product (bp)	Reference
rfbE	AAGATTGCGCTGAAGCCTTTG	467	Fortin et al. 2001
	CATTGGCATCGTGTGGACAG		
eae	TCA ATG CAG TTC CGT TAT CAG TT	482	Vidal et al. 2004
	GTA AAG TCC GTT ACC CCA ACC TG		
stx1	CAG TTA ATG TGG TGG CGA AGG	348	Cebula et al. 1995
	CAC CAG ACA ATG TAA CCG CTG		
stx2	ATC CTA TTC CCG GGA GTT TAC G	584	Cebula et al. 1995
	GCG TCA TCG TAT ACA CAG GAG C		

PCR-based analysis of *S. aureus* and *Salmonella* sp. in manures, slurries and vegetables

Primers targeting the nuc-genes (nuc-primer forw. 5' GCGATTGATGGTGATACGGTT 3' and nuc-primer rev. 5' CAAGCCTTGACGAACTAAAGC 3') and invA genes (invA-primer forw. GTGAAATTATCGCCACGTTCGGGCAA and invA-primer rev. TCATCGCACCGTCAAAGGAACC) were used in PCR reactions for detecting *S. aureus* and *Salmonella* sp., respectively, yielding PCR products of 276 bp (nuc) and 284 bp (invA) length. 25 µl PCR reactions contained 1 x PCR buffer, 2.5 mM MgCl2, 0.5 µM of each dNTP, 0.15 µM of each primer, and 0.4 U of Taq polymerase. Thermal cycling conditions for nuc PCR were: an initial denaturation at 95 °C for 5 minutes followed by 35 cycles of 95 °C for 30 sec, 53 °C for 60 sec, 72 °C for 60 sec, and a final extension at 72 °C for 10 min. Conditions for invA PCR were: an initial denaturation at 95 °C for 5 minutes followed by 40 cycles of 94 °C for 60 sec, 63 °C for 60 sec, 72 °C for 60 sec, and a final extension at 72 °C for 7 min.

PCR-based analysis of *Listeria* sp. in manures, slurries and vegetables

PCR-primers targeting the iap-gene (forward: MonoA: 5´-CAAACTGCTAACACAGCTACT-3´ ; reverse: Lis1B: 5´-TTATACGCGACCGAAGCCAAC-3´ published by Bubert et al., Appl Environ Microbiol. 1999 Oct;65(10):4688-92) enabling a species specific detection of *Listeria monocytogenes*. For the PCR the TopTaq kit (Qiagene, Hilden, Germany) was used. A 50 µl reaction contained 5 µl of 10x PCR buffer, 5 µl of 10x CoralLoad, 10 µl of 5x Q-Solution, 10 µl of 25 mM $MgCl_2$ solution, 1 µl of 200 mM of each dNTP, 0,2 µM of each Primer, 1.25 Units TopTaq DNA polymerase. The 0,66 kb PCR fragment was obtained by the use of the following touchdown PCR-Program. 5 minutes of denaturation at 94°C, followed by 35 cycles of 94°C for 30 seconds, x° C for 30 seconds, 72°C for 1 minute and a final elongation of 72°C for 10 minutes. For a higher specificity of the PCR reaction the annealing temperature was 70°C for the first cycle, and then lowered by 1°C each cycle until cycle 7. For the remaining 28 cycles the annealing temperature was 63°C.

PCR-based analysis of *Campylobacter* spp. in manures, slurries and vegetables

Primers targeting the 16S rRNA gene of Campylobacter were CampCJL1F (5'-ACT CCT TTT CTT AGG GAA GAA TTC-3') and CaArHeREV (5'-GTGGAGTACAAGACCCGGGAA-3') used in a protocol by Neubauer and Hess 2006 (J Vet Med, 53, 376-381). The PCR reactions were carried out in 25 µl volume with 10 mM Tris-HCl, 1.5 mM MgCl2, 20mM KCl, 200 µM of each dNTP, 400 pM of forward and reverse primer and 0.75 U Taq polymerase. The 946 bp PCR product was obtained by the following PCR-program: 5 min of denaturation at 94 ºC, followed by 30 cycles of 94 ºC for 2 min, 61 ºC for 1 min and 72 ºC for 1 min, and finished by a final extension of10 min at 72 ºC.

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Addendum

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

Through transnational cooperation within PathOrganic, a broader range of samples could be obtained for analysis as compared to analysis by the individual partners. As a much higher variety of different production systems was accessible for analysis as compared to those prevailing in the individual countries, a more diverse data set is available for statistical analysis and microbiological risk assessment. Including a large and diverse data set gives additional strength to the risk assessment model that will be developed within the project.

Joint expertise by the project partners was available for the development and specific adaptation of methods. During the method development phase, exchange of knowledge among the labs and training of young scientists within the consortium took place by organizing a lab workshop in succession to the 2nd consortium meeting. Furthermore, efficient and straight-forward analysis of a large number of samples was made possible by sharing specific analyses among labs according to the respective expertise.

In WP 3, individual experiments were designed and partly also set up in collaboration by multiple partners, which allowed for complementarities in expertise and technical arrangements. Since experiments were coordinated regarding their specific objectives, added scientific value was provided.

The stakeholder workshop (WP 4) was organized with contributions from all project participants. Thus, the workshop brought benefits regarding the collective expertise of the researchers. In addition, stakeholders from various countries could share their ideas and experiences based on the specific agricultural practices and regulations in their countries. Since the project outcome was of relevance for all participating countries, dissemination of the project's conclusions gained by joint research activities via the workshop and via the leaflet allowed that the project output was efficiently dispersed.

Joint research within the project gave rise to new research ideas in the field of food safety. For instance, high baseline values of pathogen infestation on the field pose the question of how post-harvest procedures and processing affect pathogen persistence and proliferation.
New research questions raised by the project include:

- Can plant breeding provide cultivars that can restrict the colonization of certain human pathogens?
- Can bacterial strains, possibly also plant growth-promoting, be inoculated to out-compete „invading" pathogens?
- Which impact do post-harvest practices have in terms of further proliferation of human pathogens already colonizing vegetable crops?
- Are there genetic markers that correlate with plant colonization traits, which might be applied in epidemiological surveillance programs?

In addition to addressing the points raised above, the project consortium has identified a need to evaluate the recommendations that were formulated based on the collective project results in the frame of follow-up experimental work.

B. Recommendations to the CORE Organic Funding Body Network in relation to launching and monitoring of future transnationally funded research projects

It is difficult to cope with the different national budgets, research priorities and financing modalities. In case several topics may be covered in one call, each country should specify the budget available for this topic.

In addition, the coordinator has only few possibilities to make financial changes, if e.g. one partner is not able to complete his task. This is because the finances are coordinated in each country individually. Therefore I suggest that the coordinator administrates the whole budget (as in EU-projects) and that he/she also has the possibility to re-allocate the budget (in case problems should arise). Such an option allows a faster problem solving process, which might be critical to the satisfactory fulfillment of tasks within a project.