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MID-TERM REVIEW REPORT

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Project acronym: VITAL

Project title: Integrated Monitoring and Control of Foodborne Viruses in European Food Supply Chains

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Periodic report: 1st 2nd 3rd 4th

Period covered: from 1st April 2008 to 31st October 2009

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Declaration by the scientific representative of the project coordinator

I, as scientific representative of the coordinator of this project and in line with the obligations as stated in Article II.2.3 of the Grant Agreement declare that:

- The attached periodic report represents an accurate description of the work carried out in this project for this reporting period;
- The project (tick as appropriate):
 - has fully achieved its objectives and technical goals for the period;
 - has achieved most of its objectives and technical goals for the period with relatively minor deviations¹;
 - has failed to achieve critical objectives and/or is not at all on schedule².
- The public website is up to date, if applicable.
- All beneficiaries, in particular non-profit public bodies, secondary and higher education establishments, research organisations and SMEs, have declared to have verified their legal status. Any changes have been reported under section 5 (Project Management) in accordance with Article II.3.f of the Grant Agreement.

Name of scientific representative of the Coordinator: ..Nigel Cook.....

Date: 06/11/2009



Signature of scientific representative of the Coordinator

¹ If either of these boxes is ticked, the report should reflect these and any remedial actions taken.

² If either of these boxes is ticked, the report should reflect these and any remedial actions taken.

1. Publishable summary



Integrated Monitoring and Control of Foodborne Viruses in European Food Supply Chains (VITAL)

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The concept of VITAL is the integrated risk assessment and management of contamination of the European farm to market food chain by pathogenic viruses. A major issue regarding foodborne viruses is the lack of effective risk management strategies and prevention and intervention measures against food and environmental contamination. The current epidemiological surveillance systems can only react to and provide information on disease outbreaks that occur through contamination of food. Such reactive surveillance alone cannot lead to any reduction in disease incidence. Decreasing the incidence and spread of foodborne viral diseases should involve prevention of food contamination in the production phase, throughout processing, during trade and distribution, and in the preparation phase, both in professional settings and in the home. In our project, we are focussing on the production and processing phase, moving away from the concept of endpoint monitoring towards input monitoring. These systems will be founded through the achievement of the four core scientific and technological objectives of VITAL.

Objective One is the acquisition of data on virus contamination of food and environmental sources. In this first project year, VITAL has produced twenty standard operating procedures for the analysis of samples from the various food supply chains which are most at risk from foodborne virus contamination. The methods underpinning these procedures were taken directly or adapted from methods available within the VITAL consortium or published in the scientific literature. With regard to the nucleic acid amplification assays, an extensive range of internal controls was constructed within VITAL, and incorporated through optimization of each assay. It is intended that

each data-gathering laboratory will use identical methodology to harmonise the data-gathering process within each food supply chain so that data can be fully comparable among and between the various food supply chains. This harmonization was initiated within the first year, through a series of ring trials of two key sample analyses – soft fruit and pork meat. The preliminary results encouraged the consideration that, with due practice, the methods will prove reliable and robust throughout VITAL's data-gathering activities. The consolidation of the elements necessary for the data-gathering activities within the VITAL consortium has established the foundation for successful achievement of the project's first Objective in the later stages of the project.

Objective Two of VITAL is the assessment of foodborne viral risks for determining high risk situations and the efficacy of interventions along the food supply chains. The data from monitoring of raw materials and food processing will be used with Modular Process Risk Models (MPRM) to build up specific hazard analysis critical control point (HACCP) recommendations. In the first 12 months of VITAL, the modules for norovirus infection due to oyster consumption have been developed. These modules jointly describe the contamination of oysters from moment of harvest through to moment of consumption. Previously collected data on norovirus contamination, and data from the scientific literature were used; this data will be replaced by VITAL data when this becomes available later in the project. VITAL has also initiated a series of studies on the survival and elimination of viruses in food production settings. These studies are being conducted within postgraduate studentships, and have been initiated with the development of experimental protocols for chlorine disinfection in a diversity of types of water and initial data on disinfection of adenoviruses has been produced. Work towards possible production of infectivity assays for hepatitis E virus and norovirus has been initiated, and will be taken forward into the next project year, where the possibilities of the initial findings will be reviewed.

The Third Objective of VITAL is to develop new measures to prevent virus contamination of foods and the environment. The data from monitoring of raw materials and food processing will be used together with the MPRMs to build up specific HACCP recommendations. These recommendations will be incorporated in a Code of Good Practice, and in the last two months of this first project year, work towards the production of this Code began with identification of what information exists in the literature from the last 40 years regarding virus contamination and food safety. From the very initial impressions gained from this survey, in regard to legislation, regulation, inspection, education/training etc. it is very apparent that recognition of the hazards associated with virus contamination of foods is scant. The VITAL Code of good Practice is intended to remedy this, and its preparation will be assiduously progressed in the next stages of the project.

VITAL's Fourth Objective is to develop and assess measures for virus reduction and control in case of virus contamination. These measures will finally be recommended in the Code of Good Practice. Towards this end, VITAL will undertake an extensive review of current HACCP procedures practiced by food chain operators. In the project, data gathering on current HACCP and other food safety systems is using two approaches. The first approach is a bench study of information available on the Internet and in scientific literature. The second approach has been the formulation of background review questionnaires to gather generic and specific information on food process flow charts, food safety systems in place, assessment of CCP's, prerequisite conditions, etc. from partners within the VITAL consortium which they have obtained from liaison with local food chain operators. The first background review questionnaires were directed towards the production stages of soft fruit, salad vegetables, and pork, and the processing of soft fruit and salad vegetables. Analysis of returned background review questionnaires is still underway. The background review questionnaires will be followed up in the next project year by a series of fact-finding missions, to the selected food chain operators in each of the countries from which VITAL will perform monitoring for virus contamination. The agreements with the operators have been negotiated in the first project year.

The outcomes of VITAL must be of value to Europe. To ensure this, VITAL intends to consolidate and deliver its findings by publishing its Code of Good Practice, and present to the appropriate stakeholders the requirements necessary for establishing reliable monitoring of food chains for viruses on a regular or as-needed basis, which will include the tried and tested SOPs from the project. VITAL will ultimately provide to Europe a framework for monitoring, risk modeling, and procedures for control of foodborne virus contamination, the implementation of which should form a first line of defence against transmission of foodborne viral diseases in Europe.

2. Project objectives for the period

In this first project year, there was only one Milestone: M2.1 “All data-gathering laboratories fully prepared with necessary materials and SOPs”. This milestone was scheduled in the Description of Work to be a step towards VITAL Objective 1 “To acquire data on virus contamination of food and environmental sources”. Due to the unanticipated lack of availability of standard methods for food analysis, detailed SOPs had to be devised and tested within VITAL itself, and this required several additional months for Task 2.1 “Preparatory activities”. All SOPs were prepared by Month 12, and ring trials of key methods were planned, and trial materials prepared and sent to participants, by Month 12. The ring trials were completed by Month 15, and thus Milestone M2.1 has been met. The arrival at this milestone ensures that the data-gathering laboratories will use standardised detection methods for virus detection throughout the selected food supply chains from farm to market. The data acquired will then be suitable for quantitative viral risk assessment (QVRA), in the subsequent stages of the project. Thus, good progress has been made towards fulfilling the Objectives of VITAL.

3. Work progress and achievements during the period

3.1 The progress of WP1 “Project Management”, is detailed in Section 5 below.

3.2 Report on Workpackage 2 “Data-Gathering: Production” progress and achievements during the period 1st April 2008 to 31st March 2009

Start date or starting event: Month 4

T2.1 Preparatory activities. During the preparation of the proposal, it was envisaged that standard operating procedures (SOPs) for extraction and detection of viruses from food and related samples would be available from the CEN TAG4 group in early 2008. However, during the 1st Consortium Meeting, information obtained from TAG 4 revealed that these SOPs would not be published until 2012. It was therefore agreed to draft dedicated VITAL SOPs for virus detection in the food production chains.

The WP leader in liaison with ITACyL (Partner 12) compiled the current approaches used for detection of pathogenic and surrogate virus in the food matrixes described in the research proposal in order to guarantee that the previous experience on detection of foodborne viruses and application of molecular methods in food microbiology was totally gathered and exploited. Afterwards, a round of discussions was held with expert labs in the topic within the VITAL Consortium advised by the VITAL External Committee for the selection of the most sensible and sound methodological approaches. Once the approaches were selected for each food matrix and analytical step -sampling and virus concentration, nucleic acid extraction, and (RT-)PCR detection-, an working strategy was devised: The first draft of each SOP was developed by ITACyL, revised by the WP2 leader and release to the VITAL Consortium for suggestions and/or modifications. Subsequently all the suggestions and/or modifications were included in the 2nd version of the SOPs, and these were revised and accepted consecutively by DEFRA (Partner 1) and WP2 leader. Once the final version was accepted it was again released to the VITAL Consortium for further comments, and if any these were included in subsequent versions. In parallel, ITACyL assessed the applicability of each the SOPS in the lab and the WP2 leader also requested to Data Gathering laboratories (partners 3, 4, 5, 6, 9, 10 and 12) to assess the applicability in each single laboratory. After this practical round of evaluations, the final version of the SOPs could be finished, and presented in the 2nd Annual VITAL Consortium Meeting in Novi Sad.

Due to the development and testing of project-specific new SOPs milestone M2.1 was reset to Month15.

The VITAL SOPs are listed in Table 1.

Table 1. The VITAL SOPs

Title of SOP	Number
Sampling and virus concentration from faeces	001
Sampling and virus concentration from harvesters' hands	002
Sampling and virus concentration from animal-derived fertilizer	003
Sampling and virus concentration from waters	004
Sampling and virus concentration from soft fruit	005
Sampling and virus concentration from vegetables	006
Sampling and virus concentration from shellfish	007
Sampling and virus concentration from blood	008
Sampling and virus concentration from pork meat and liver tissue	009
Nucleic acids extraction from faeces, animal-derived fertilizer, or blood	010
Nucleic acids extraction from pork liver tissue or meat	011
Nucleic acids extraction from soft fruits, vegetables or shellfish	012
Nucleic acids extraction from irrigation water, slaughterhouse effluents, or harvesters' hands wash-off	013
General adenovirus QPCR*	014
Standard Operating Procedure for detection and quantification of porcine adenoviruses by real-time PCR †	015

Human adenovirus nested PCR	017
Detection and quantification of norovirus by real-time reverse transcription PCR	018
Detection and quantification of hepatitis A virus by real-time reverse transcription PCR	019
Detection and quantification of hepatitis E virus by real-time reverse transcription PCR	020
Detection and quantification of murine norovirus by real-time reverse transcription PCR	021

* From EU FP6 project 513648 “Methods for the concentration and detection of adenoviruses and noroviruses in european bathing waters with reference to the revision of the bathing water directive 76/160/EEC (VIROBATHE)”.

† From the University of Barcelona, by MTA.

At the end of 2008 all drafted SOPs were made available to the beneficiaries through the VITAL website. From that time on beneficiaries could start practicing these protocols.

At the 1st Consortium Meeting the inclusion of internal amplification controls (IACs; Hoorfar et al., 2003) was agreed, and a specific IAC for each real-time (RT) PCR assay was designed, developed and assessed. After confirmation of the correct performance in duplex format with the virus-specific PCR system, the control materials were sent to a Biotechnology Company (Yorkshire BioScience) for large-scale preparation, before being made available to the VITAL participant laboratories.

To be able to determine whether the extraction procedures prior to application of the (RT)PCRs have performed correctly in the diverse matrices, it is necessary to use a sample process control (SPC), which is a non-target virus spiked into each sample and detected separately but simultaneously (Croci et al., 2008). Initially, it was intended to use the SPC virus proposed by the TAG4 group – a modified strain of mengovirus – but it was learnt that this virus is a genetically modified organism, and requires a specific licence for handling and containment. Due to this constraint therefore, it was decided to find another more suitable virus for use as the VITAL SPC. Several candidates were discussed, and it was decided to use the recently discovered murine norovirus (Wobus et al., 2006). This virus was obtained through Washington University, by MTA (see p 38), and distributed among the project partners.

To estimate the laboratory and method performances of the data gathering laboratories, and the robustness of the performance characteristics of selected key methods a series of ring trials was planned. The methods selected for trial were the detection of virus in soft fruit (SOPs 5 and 12), and the detection

of virus in pork products (SOPs 9 and 11). Human adenovirus serotype 5 was selected to be used to test each method (SOP 17) and each method would incorporate the use of the SPC (detected by SOP 21). Four rounds of pre-collaborative trial were planned, before the main ring trial. These pre-collaborative trials are “dry runs” of the ring trial to test the robustness of VITAL SOP 009 (“Sampling and virus concentration from pork meat”), and should help to identify any areas where modification or clarification of the ring trial protocol may be necessary. In this reporting period, two pre-collaborative trials were performed. SOPs for these pre-collaborative trials are shown in Annex 2 of this Report.

The stocks of human Adenovirus serotype 5 (HAdV-5) and Murine Norovirus genogroup V (MNV-1) strains to be used during the ring trials and as control viruses during the following detection phases of the VITAL project were obtained from the University of Barcelona and the Washington University Medical School of St. Louis, respectively. Viruses were propagated for 6 sequential passages in cultures of A549 and RAW cells, respectively, to reach work viral concentrations. Viruses were titrated by both viral plaque and tissue culture 50% CPE assays, yielding titers of approximately 4×10^7 and 10^8 TCID₅₀ per ml of work suspension stocks for Had-5 and MNV-1, respectively. Part of the produced viral suspensions were used for establishing the control reagents to be used in the ring trials, subsequently supplied to partners participating in the experiments, and part were used for production of control preparations of viral genomic DNA and RNA, respectively. These nucleic acid suspensions, purified in bulk, were adjusted for use in real-time (RT) PCR assays, and shipped to all partner laboratories. All test materials for the pre-collaborative trials and the ring trials were prepared at ISS, and each participant laboratory received individually coded samples of the test materials (see Annex 2), and individualised SOPs. In VITAL, all participating institutes take part in the trials, except for KULeuven and UL-BF. Thus, all the data-gathering institutes plus the institutes in which the VITAL studentships are taking place, will contribute data to the trials; this gives a total of 11 laboratories, which will increase the statistical value of the determination of the methods’ robustness.

The results of the pre-collaborative trials 1 and 2 are shown in the tables 2 and 3 below. The results are given in terms of number of correct identifications rather than reporting individual CT values

Table 2. Results for pre-collaborative trial 1 and 2 for soft fruit (raspberries)

	Trial 1	Trial 2
	Correct	Correct
Blank‡	16 / 18	15 / 18
Adenovirus “Low” †	16 / 18	18 / 18
Adenovirus “High” †	18 / 18	18 / 18
SOP 14 non-template control‡	18 / 18	18 / 18
Murine norovirus‡	36 / 48	45 / 48
SOP 21 non template control*	16 / 16	16 / 16

‡Nine laboratories reported results, performing each test in duplicate.

‡Eight laboratories reported results, performing each test six times

*Eight laboratories reported results, performing each test in duplicate.

Table 3. Results for pre-collaborative trial 1 and 2 for pork meat (cooked ham)

	Trial 1	Trial 2
	Correct	Correct
Blank‡	20 / 20	15 / 20
Adenovirus “Low” †	15 / 20	15 / 20
Adenovirus “High” †	17 / 20	18 / 20
SOP 14 non-template control‡	20 / 20	18 / 20
Murine norovirus‡	26 / 54*	31 / 42**
SOP 21 non template control	18 / 18*	14 / 14**

‡Ten laboratories reported results, performing each test in duplicate.

‡Eight laboratories reported results, performing each test six times

*Nine laboratories reported results, performing each test in duplicate.

**Seven laboratories reported results, performing each test in duplicate.

The results indicate that, upon first implementation and practice, the methods are working robustly in the participant laboratories. There is an indication that in some cases, during sample treatment, contamination with target virus (adenovirus) may have occurred, possibly through cross-contamination between the blank and the spiked samples. This highlights the need for the analytical laboratories to be very scrupulous when performing these tests. Overall, these preliminary results encouraged the consideration that, with due practice, the methods will prove reliable and robust throughout VITAL’s data-gathering activities.

T2.2 Data-gathering: salad vegetable farms

In Poland one lettuce farm was selected and one farm was selected which produces different kinds of vegetables such as lettuce, spring onion, radish, cabbage.

In Serbia one large production enterprise was selected (which supplies one big processing premise in Serbia as well as the biggest retailers in Belgrade) and 3 smaller production enterprises were selected (small family business productions which supply farm markets in their surroundings).

In Greece, one very big and organized farm (having HACCP) which produces several kinds of vegetables and fruits were selected and one smaller family farm was selected which produces different kind of vegetables such as lettuce, spring, onions.

Table 4. Salad vegetable farms identified for future sampling in VITAL

Country	Number of salad vegetable farms identified for future sampling
Greece	2
Poland	2
Serbia	4

T2.3 Data-gathering: soft fruit farms.

In the Czech Republic three strawberry farms were selected. In Finland one selected company is packing frozen berries. Another quite small (about 25 workers from Ukraine and Latvia) farm growing strawberries and raspberries, is willing to participate. This farm may use irrigation for strawberries, but never for raspberries.

In Serbia 1 large production enterprise was selected (which supplies the biggest retailers in Serbia and exports fruits to Belgium and the UK), and 3 small family business production enterprises were selected (which supply retailers with refrigerated warehouse, and other retailers and markets in Serbia)

In Poland two raspberries plantations were selected. Raspberries produced on these farms are mostly processed in local processing unit. Only small amount is sold as fresh produce to retailers.

Table 5. Soft fruit farms identified for future sampling in VITAL

Country	Number of soft fruit farms identified for future sampling
Czech Republic	3
Finland	1
Poland	2
Serbia	4

T2.4 Data-gathering: pig farms.

This data will be gathered with reference to the vaccination study (see T6.4). In the Czech Republic 3 large pig farms were selected (more than 500 sows). In The United Kingdom and Spain fattening pig farms were approached for sample collection. In Italy, large farms were approached for sampling within the associates of a major national company.

Table 6. Pig farms identified for future sampling in VITAL

Country	Number of pig farms identified for future sampling
Czech Republic	3
Italy	2
Spain	5
United Kingdom	2
Netherlands	1

T2.5 Data-gathering: slaughterhouses.

Table 7. Slaughterhouses identified for future sampling in VITAL

Country	Number of slaughterhouses identified for future sampling
Czech Republic	2
Italy	2
Spain	4
United Kingdom	4

References

Luciana Croci, Eric Dubois, Nigel Cook, Dario de Medici, Anna Charlotte Schultz, Bernard China, Saskia Rutjes, Jeffrey Hoorfar, Wim H.M. Van der Poel (2008) Methods for extraction and concentration of enteric Viruses from fresh fruit and vegetables. *Food Analytical Methods* 1:73-84.

Hoorfar J, Cook N, Malorny B, Wagner M, De Medici D, Abdulmawjood A, Fach P.. (2003) Making internal amplification control mandatory for diagnostic PCR. [J Clin Microbiol.](#) 2003 Dec;41(12):5835.

Wobus CE, Thackray LB, Virgin HW 4th. Murine norovirus: a model system to study norovirus biology and pathogenesis. (2006). *J. Virol.* 80 5104-12.

3.2.1 VITAL WP2 Mid-Term Update (April 1st- October 31st, 2009)

In the period of April 1 to October 31st, 2009 of the project within WP2 the work mainly included task 2.1, the preparatory work. Drafting of SOPs was continued and reference materials were constructed (especially by partner 12, ITACyL, and supported by partner 6, ISS) Amendments to already drafted SOPs were included. In addition to already existing SOPs, SOP 16 on bovine polyomavirus, SOP22 on quality controls and SOP 23 on porcine adenovirus IAC were finalized. In Table 17 there is an updated list of the VITAL SOPS.

All SOPs have been made available for the beneficiaries through the VITAL website. In order to enable all beneficiaries to practice to work according to updated final SOPs, reference materials were prepared by Partner 6 (ISS Rome) for murine norovirus and for human adenovirus.

Ring trial of the real-time PCR-based method for analysis of soft fruit

Each participant was provided with a personalised SOP for performance of this trial. Coded vials containing virus suspensions were sent to each participant. For preparation of the ring-trial samples, fresh raspberries were purchased separately by each participant from local sources. Three portions comprising 25 g raspberries were artificially contaminated with human adenovirus at 5×10^4 pfu (high). Three portions comprising 25 g raspberries were artificially contaminated with human adenovirus at 5×10^2 pfu (low). Three portions comprising 25 g raspberries were not artificially contaminated (blank). All portions were spiked with the sample process control, murine norovirus, at 5×10^5 . Viruses were extracted from all samples using SOP 005 “Sampling and virus concentration from soft fruit” and nucleic acids were extracted from the soft fruit extract using SOP 012 (“Nucleic Acids Extraction from soft fruits, vegetables or shellfish”). Real-time PCRs for human adenovirus (SOP 014 “General adenovirus QPCR”) and murine norovirus (SOP 021 “Detection and quantification of murine norovirus by real-time reverse transcription PCR”) were performed in duplicate for all samples, using the extracted nucleic acids as template. Raw data (not shown) was reported by each participant to the trial leader, who translated the codes and analysed the data.

Table 8 gives the participants results of the analysis of the “HIGH” artificially contaminated soft fruit samples.

Table 8 Participants results of the analysis of the “HIGH” artificially contaminated soft fruit samples

	Sample A					Sample B					Sample C				
	HAdV		MNoV		Int.	HAdV		MNoV		Int.	HAdV		MNoV		Int.
	Rep. 1	Rep. 2	Rep. 1	Rep. 2		Rep. 1	Rep. 2	Rep. 1	Rep. 2		Rep. 1	Rep. 2	Rep. 1	Rep. 2	
VLA	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
VRI	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
UH	+	+	+	+	C	F	+	-	+	C	F	F	-	-	AF
UPA	+	+	-	-	C	+	+	-	+	C	+	+	-	+	C
ISS	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
RIVM	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
WUR	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
NVRI	+	+	F	F	C	+	+	F	F	C	+	+	F	F	C
NIV-NS	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
UB	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
ITACyL	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C

Rep. = Replicate real-time PCR; Int = Interpretation; “+” = Target signal present, IAC signal present OR absent; “-“ = Target signal absent, IAC signal present; “F” = Target signal absent, IAC signal absent
“C” = Sample contaminated; “AF” = Analysis Failed.

Samples were interpreted as contaminated (C) if at least one of the replicates was positive for HAdV.

Table 9 gives the participants results of the analysis of the “LOW” artificially contaminated soft fruit samples.

Table 9 Participants results of the analysis of the “LOW” artificially contaminated soft fruit samples.

	Sample A					Sample B					Sample C				
	HAdV		MNoV		Int.	HAdV		MNoV		Int.	HAdV		MNoV		Int.
	Rep. 1	Rep. 2	Rep. 1	Rep. 2		Rep. 1	Rep. 2	Rep. 1	Rep. 2		Rep. 1	Rep. 2	Rep. 1	Rep. 2	
VLA	+	+	+	+	C	+	+	+	+	C	+	+	+	-	C
VRI	+	+	+	+	C	+	+	+	+	C	+	-	+	+	C
UH	+	+	-	-	C	+	+	-	-	C	+	+	F	-	C
ISS	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
RIVM	+	+	+	+	C	+	+	+	+	C	+	+	F	+	C
WUR	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
NVRI	+	+	F	F	C	+	+	F	F	C	+	+	F	F	C
NIV-NS	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
UB	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
ITACyL	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C

Rep. = Replicate real-time PCR; Int = Interpretation; “+” = Target signal present, IAC signal present OR absent; “-“ = Target signal absent, IAC signal present; “F” = Target signal absent, IAC signal absent
 “C” = Sample contaminated; “AF” = Analysis Failed.

Samples were interpreted as contaminated (C) if at least one of the replicates was positive for HAdV.

Table 10 gives the participants results of the analysis of the non artificially contaminated soft fruit samples.

Table 10 Participants results of the analysis of the non artificially contaminated soft fruit samples.

	Sample A					Sample B					Sample C				
	HAdV		MNoV		Int.	HAdV		MNoV		Int.	HAdV		MNoV		Int.
	Rep. 1	Rep. 2	Rep. 1	Rep. 2		Rep. 1	Rep. 2	Rep. 1	Rep. 2		Rep. 1	Rep. 2	Rep. 1	Rep. 2	
VLA	-	-	+	+	UC	-	-	+	+	UC	-	-	+	+	UC
VRI	-	-	+	+	UC	-	-	+	+	UC	-	-	+	+	UC
UH	+	+	+	+	C	-	-	+	+	UC	F	F	-	-	AF
UPA	+	-	F	-	C	-	-	-	-	AF	-	-	-	-	AF
ISS	-	-	+	+	UC	-	-	+	+	UC	-	+	+	+	C
RIVM	-	-	+	+	UC	-	-	+	+	UC	-	-	+	+	UC
WUR	-	-	+	+	UC	-	-	+	+	UC	-	-	+	+	UC
NVRI	F	F	F	F	AF	F	F	F	F	AF	F	F	F	F	AF
NIV-NS	-	-	+	+	UC	-	-	+	+	UC	+	+	+	+	C
UB	-	F	+	+	UC	-	F	+	+	UC	F	-	+	+	UC
ITACyL	-	-	+	+	UC	-	-	+	+	UC	-	-	+	+	UC

Rep. = Replicate real-time PCR; Int = Interpretation; “+” = Target signal present, IAC signal present OR absent; “-“ = Target signal absent, IAC signal present; “F” = Target signal absent, IAC signal absent “C” = Sample contaminated; “AF” = Analysis Failed; “UC” = sample not contaminated.

Samples were interpreted as uncontaminated (UC) if BOTH replicates were negative for HAdV, and at least one replicate was positive for MNoV, the sample process control. Samples were interpreted as contaminated (C) if at least one of the replicates was positive for HAdV. UB’s Samples A, B and C were interpreted as uncontaminated (UC) because one of their replicates was negative for HAdV, and the other failed, and both replicates were positive for MNoV, the sample process control, indicating the extraction worked correctly.

Table 11 shows the correctly identified samples in the soft fruit ring trial.

Table 11 The correctly identified samples in the soft fruit ring trial

Contamination level	Samples Analysed	Correctly identified	% correctly identified
High	33	32	97.0
Low	30	30	100
Not contaminated	33	23	69.7

Partners with failed reactions have been advised to ensure that their IACs are included henceforward in their reactions at the correct concentration. Four of the non-artificially contaminated samples gave positive for HadV results. It could be instructive to sequence the amplicons to check whether the contamination was due to cross-contamination with the “HIGH” or “LOW” samples, or whether it was naturally occurring.

These overall results were considered to show that the real-time PCR-based methods for detection of viruses in soft fruits were acceptably robust and therefore that the data-gathering should commence.

Ring trial of the real-time PCR-based method for analysis of pork products

Each participant was provided with a personalised SOP for performance of this trial. Coded vials containing virus suspensions were sent to each participant. For preparation of the ring-trial samples, pork livers were purchased separately by each participant from local sources. Three portions comprising 250 mg pork liver were artificially contaminated with human adenovirus at 5×10^4 pfu (high). Three portions comprising 250 mg pork liver were artificially contaminated with human adenovirus at 5×10^2 pfu (low). Three portions comprising 250 mg pork livers were not artificially contaminated (blank). All portions were spiked with the sample process control, murine norovirus, at 5×10^5 pfu. Viruses were extracted from all samples using SOP 009 “Sampling and virus concentration from pork meat and liver tissue” and nucleic acids from the liver extract by SOP 011 (“Nucleic acids extraction from pork liver tissue or meat”). Real-time PCRs for human adenovirus (SOP 014 “General adenovirus QPCR”) and murine norovirus (SOP 021 “Detection and quantification of murine norovirus by real-time reverse transcription PCR”) were performed in duplicate for all samples, using the extracted nucleic acids as template. Raw data (not shown) was reported by each participant to the trial leader, who translated the codes and analysed the data.

Table 12 gives the participants results of the analysis of the “HIGH” artificially contaminated pork product samples.

Table 12 Participants results of the analysis of the “HIGH” artificially contaminated pork product samples.

	Sample A				Int.	Sample B				Int	Sample C				Int.
	HAdV		MNoV			HAdV		MNoV			HAdV		MNoV		
	Rep. 1	Rep. 2	Rep. 1	Rep. 2		Rep. 1	Rep. 2	Rep. 1	Rep. 2		Rep. 1	Rep. 2	Rep. 1	Rep. 2	
VLA	-	+	+	+	C	+	+	+	+	C	+	+	+	+	C
VRI	+	+	-	-	C	+	+	-	+	C	+	+	+	+	C
UH	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
ISS	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
RIVM	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
WUR	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
NVRI	+	+	F	F	C	+	+	F	F	C	+	+	F	F	C
NIV-NS	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
UB	-	F	+	+	UC	+	+	+	+	C	+	+	+	+	C
ITACyL	+	+	-	-	C	+	+	-	-	C	+	+	-	-	C

Rep. = Replicate real-time PCR; Int = Interpretation; “+” = Target signal present, IAC signal present OR absent; “-” = Target signal absent, IAC signal present; “F” = Target signal absent, IAC signal absent
 “C” = Sample contaminated; “AF” = Analysis Failed; “UC” = sample not contaminated.

Samples were interpreted as contaminated (C) if at least one of the replicates was positive for HAdV.

UB’s Sample A was interpreted as uncontaminated (UC) because one of the replicates was negative for HAdV, and the other failed, and both replicates were positive for MNoV, the sample process control, indicating the extraction worked correctly.

Table 13 gives the participants results of the analysis of the “LOW” artificially contaminated pork product samples.

Table 13 Participants results of the analysis of the “LOW” artificially contaminated pork product samples

	Sample A					Sample B					Sample C				
	HAdV		MNoV		Int.	HAdV		MNoV		Int.	HAdV		MNoV		Int.
	Rep. 1	Rep. 2	Rep. 1	Rep. 2		Rep. 1	Rep. 2	Rep. 1	Rep. 2		Rep. 1	Rep. 2	Rep. 1	Rep. 2	
VLA	+	+	+	+	C	+	+	-	-	C	+	+	+	-	C
VRI	+	+	-	-	C	+	+	-	-	C	+	+	-	-	C
UH	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
ISS	+	+	+	-	C	+	+	+	+	C	+	+	+	+	C
RIVM	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
WUR	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
NVRI	+	+	F	F	C	+	+	-	-	C	+	+	F	F	C
NIV-NS	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
UB	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
ITACyL	+	+	-	-	C	+	+	-	-	C	+	+	-	-	C

Rep. = Replicate real-time PCR; Int = Interpretation; “+” = Target signal present, IAC signal present OR absent; “-“ = Target signal absent, IAC signal present; “F” = Target signal absent, IAC signal absent
 “C” = Sample contaminated; “AF” = Analysis Failed.

Samples were interpreted as contaminated (C) if at least one of the replicates was positive for HAdV

Table 14 gives the participants results of the analysis of the non artificially contaminated pork product samples

Table 14 Participants results of the analysis of the non artificially contaminated pork product samples

	Sample A					Sample B					Sample C				
	HAdV		MNoV		Int.	HAdV		MNoV		Int.	HAdV		MNoV		Int.
	Rep. 1	Rep. 2	Rep. 1	Rep. 2		Rep. 1	Rep. 2	Rep. 1	Rep. 2		Rep. 1	Rep. 2	Rep. 1	Rep. 2	
VLA	-	-	+	F	UC	-	-	-	+	UC	-	-	+	+	UC
VRI	-	-	+	-	UC	-	-	-	-	AF	-	-	-	-	AF
UH	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
ISS	+	+	+	+	C	+	+	+	+	C	-	-	+	+	UC
RIVM	-	-	+	+	UC	-	-	+	+	UC	-	-	+	+	UC
WUR	-	-	-	+	UC	-	+	+	+	C	-	+	+	+	C
NVRI	+	+	-	-	C	+	+	-	-	C	+	+	-	+	C
NIV-NS	+	+	+	+	C	-	+	+	+	C	-	-	+	+	UC
UB	-	-	+	+	UC	-	-	+	+	UC	-	-	+	+	UC
ITACyL	-	-	-	-	AF	-	-	-	-	AF	-	-	-	-	AF

Rep. = Replicate real-time PCR; Int = Interpretation; “+” = Target signal present, IAC signal present OR absent; “-“ = Target signal absent, IAC signal present; “F” = Target signal absent, IAC signal absent “C” = Sample contaminated; “AF” = Analysis Failed; “UC” = sample not contaminated.

Samples were interpreted as uncontaminated (UC) if BOTH replicates were negative for HAdV, and at least one replicate was positive for MNoV, the sample process control. Samples were interpreted as contaminated (C) if at least one of the replicates was positive for HAdV.

Table 15 shows the correctly identified samples in the pork product ring trial.

Table 15 The correctly identified samples in the pork product ring trial.

Contamination level	Samples Analysed	Correctly identified	% correctly identified
High	30	29	96.7
Low	30	30	100
Not contaminated	30	13	43.3

Twelve of the non-artificially contaminated samples gave positive for HAdV results. It will be necessary to sequence the amplicons to check whether the contamination was due to cross-contamination with the “HIGH” or “LOW” samples, or whether it was naturally occurring due to contamination of the pork livers with porcine adenovirus. Partners with failed reactions have been advised to ensure that their IACs are included henceforward in their reactions at the correct concentration.

Tasks 2.2 – 2.5

Data gathering labs (partners 3 VRI, 4 UH, 5 UPA, 9 NVRI, 10 NIV-NS, 12 ITACyL) have started to collect samples, mainly from fruit and vegetable farms of which sampling was started by all data gathering labs. Sample collection from slaughterhouses has been started in just a few labs. For the latter sampling most of the partners are still waiting for the fact-finding missions visits which are to be done prior to the sampling. In Table 16 the numbers of collected samples are listed.

Table 16 Collected samples in WP2 at Mid-Term.

WP	Task	Number of samples taken up to October 31 st 2009	Present status
2	T2.2 Data-gathering: salad vegetable farms.	100	samples extracted and stored at -80C or -20C as appropriate.
	T2.3 Data-gathering: soft fruit farms.	212	
	T2.5 Data-gathering: slaughterhouses.	46	

Table 17 Updated list of VITAL SOPs at Mid-Term.

Title of SOP	Number
Sampling and virus concentration from faeces	001
Sampling and virus concentration from harvesters' hands	002
Sampling and virus concentration from animal-derived fertilizer	003
Sampling and virus concentration from waters	004
Sampling and virus concentration from soft fruit	005
Sampling and virus concentration from vegetables	006
Sampling and virus concentration from shellfish	007
Sampling and virus concentration from blood	008
Sampling and virus concentration from liver tissue and pork meat	009
Nucleic acids extraction from faeces, animal derived fertilizer, or blood	010
Nucleic acids extraction from pork liver tissue or meat	011
Nucleic acids extraction from soft fruits, vegetables or shellfish	012
Nucleic acids extraction from irrigation water, slaughterhouse effluents, or harvesters' hands wash-off	013
General adenovirus QPCR (From VIROBATHE project)	014
Standard Operating Procedure for detection and quantification of porcine adenoviruses (from UB)	015
Bovine polyomavirus QPCR	016
Human adenovirus nested PCR	017
Norovirus ggII	018
Norovirus ggI	019
HAV QPCR	020
HEV QPCR	021
SPC QPCR	022
Porcine adenovirus IAC	023

3.3 Report on Workpackage 3 “Data-Gathering: Processing” progress and achievements during the period 1st April 2008 to 31st March 2009

Start date or starting event: Month 4

The objectives of WP3 are to gather data on virus presence in pork-processing plants, soft fruit-processing plants and in salad vegetables-processing plants. The work for this workpackage started, together with WP2 and WP4, at month 4 as agreed at the kick-off meeting in Brno in April 2008, but as the time period for the method introduction and ring trials has been prolonged, the samples of WP3 will be taken from summer 2009 onwards.

The task leaders for data gathering have been selected:

T3.1. Fruit processing Tamas Petrovic (NIV-NS)

T3.2. Vegetables Artur Rzezutka (NVRI)

T3.3. Pork Franco Ruggeri (ISS)

The detailed plans for sampling regarding processing have been agreed at several RDMB and ad-hoc meetings during the first year.

The SOPs for the analysis of the samples will be those, common to WP2 and WP4, which have been produced in task T2.1.

Each data-gathering participant provisionally identified suitable relevant processing plants in their countries, then negotiated with many of them in order to finally identify preferably 3 processing plants in the food supply chain they will sample. Each data-gathering participant has made a time-schedule for sampling at the processing plants. All partners have either already identified 1-3 companies or the negotiations are underway, which means that the time-schedule expected has been kept well. It was not always possible for each country to find 3 processing plants willing to participate in the project, but at least one plant has been identified by data-gathering participants. It looks like not all soft fruit supply chains include a processing stage. If this is the case, then the number of samples for processing will be divided between the samples taken at the production and point of sale stages. Preliminarily, one sampling point for processing of salad vegetables is the water used for washing and for processing of

pork is the meat mincer. The final sampling points at the processing plants will be determined based on the filled background review questionnaires.

T3.1 Data-gathering: Soft fruit processing.

For soft fruit processing, all participants have identified one candidate. All of them are processing, - mainly freezing, storing and packaging – berries such as straw- or raspberries. Poland, Serbia and the Czech Republic represent countries that produce most of the European berries and mostly process domestic berries, whereas Finland produces berries mainly for domestic use, but processes a lot of imported berries as well.

Table 18. Soft fruit processors identified for future sampling in VITAL

<i>Country</i>	<i>Number of soft fruit processors identified for future sampling</i>
Czech Republic	1
Finland	1 (packing domestic and imported frozen berries)
Poland	1 (the manufacturing plant and the cold store)
Serbia	1 big processing unit – supplies the biggest retailers in Serbia as well as exports the fruits to Belgium and UK

T3.2 Data-gathering: Salad vegetable processing.

All data-gathering participants have made inquires concerning vegetable processing. In many cases (e.g. Poland) it has been found to be closely connected to production. Processing plants representing both small- and large-scale processing have been included.

Table 19. Salad vegetable processors identified for future sampling in VITAL

Country	Number of salad vegetable processors identified for future sampling
Greece	2 premises with small scale processing immediately after harvesting
Poland	2 (there is only on-farm processing involved)
Serbia	1 (big processing premises – products from different producers – supplies the McDonalds in Serbia as well as the biggest retailers in Belgrade) and another 3 (where there is only limited on-farm processing involved)

T3.3 Data gathering: Pork processing.

One of the goals of VITAL is to estimate the foodborne risks for viruses using Quantitative Viral Risk Assessment (QVRA). In case of hepatitis E virus (HEV), this risk may be posed by consumption of pork meat. It is now known from previously published data that the highest prevalence of HEV in pigs is seen at the age of two to four months. At slaughter, when pigs are approximately six months of age, the prevalence is known to be lower. Thus, using the prevalence of HEV on pig farms for QVRA will overestimate the prevalence and consequently the associated risk of consuming meat from pigs. Samples taken at slaughterhouses will gain a more accurate insight into the possible risk of foodborne HEV infections in humans due to pork consumption. Therefore, the samples for VITAL's QVRA will be collected at slaughterhouses instead of on pig farms. In some cases negotiations with processing companies have been time-consuming and they are still in progression (e.g. in Italy). Several pork processing plant candidates have been identified by Czech, Spain and United Kingdom.

Table 20. Pork processors identified for future sampling in VITAL

Country	Number of pork processors identified for future sampling
Czech Republic	2 (slaughterhouses)
Italy	1 company with several sites*
Spain	2 companies contacted (preliminary sampling): one farm-slaughterhouse and meat processing plant, one meat processing plant (in addition, permanent contacts with 2 slaughterhouses).
United Kingdom	4 (slaughterhouses)

*Italy: Samplings at all steps during the pork food chain, i.e. production, slaughter, processing and point of sales, will all be carried out at the premises of companies linked with the Coop-Italia, in Emilia-Romagna. A Secrecy Agreement between Coop-Italia, the Istituto Superiore di Sanità, and the University of Bologna - Subcontractor of ISS for sampling - has been written to this aim, approved by the legal offices of institutes involved, and has been forwarded for the official signature by the Authorized Representatives of the institutions. This act will ensure the optimal selection of sampling sites, likely along a same local chain from production to retail, as well as the access of the Partner Unit members to the sites themselves. Due to different final products in distinct sites, processing and point of sales sites will be chosen after items to be sampled have been conclusively defined."

3.3.1 VITAL WP3 Mid-Term Update (April 1st- October 31st, 2009)

Data gathering laboratories took the first samples in WP3 during this time period. In total, 119 samples were collected linked to soft fruit processing. Most samples, all linked to raspberry processing, were taken by Poland (63 samples) and Serbia (49 samples), Finland collected 7 samples. The processing of soft fruit in Finland included especially storing, freezing and packaging of berries, after which the berries were delivered to retail and/or points of sale. Mainly swab samples were taken. Czech Republic was waiting for fact-finding mission and was not able to start sample collection.

In task T3.2, a total of 49 salad or vegetable samples were collected by all participants joining this task, Greece (20 samples), Poland (21 samples) and Serbia (8 samples). The pork processing was still waiting for fact-finding mission to be completed and thus no samples were collected for task T3.3. In pork meat processing plants identified by VLA the meat was cut and packaged for distribution to the points of sale. The fact-finding visit and the samplings will be performed before the end of February 2010.

For all samples, sample extraction was performed and the extracts were stored at -20°C or -80°C according to the SOPs. The virus analyses will be performed as soon as the final virus standards for gene amplification are available.

A summary of the data regarding total amount of samples taken for each task are presented in the Table 21.

Table 21 Collected Samples in WP3 at Mid-Term

WP	Task	Number of samples taken*	Present status
3	T3.1 Data-gathering: Soft fruit processing	119	Samples extracted and stored -20 or -80°C as appropriate
	T3.2 Data-gathering: Salad vegetable processing.	49	
	T3.3 Data gathering: Pork processing.	0	
	Total	168	

* Includes ad-hoc samples

3.4 Report on Workpackage 4 “Data-Gathering: Point of Sale” progress and achievements during the period 1st April 2008 to 31st March 2009

Start date or starting event: Month 4

Work schedule and sampling arrangements

In the work schedule agreed for VITAL, food samples analysis within WP4 should commence during last year of the project realisation. Due to changes made in the work plan of the project, each partner is responsible for an arrangement of own sampling schedule for WP4, which will not interact with the work carried out for other workpackages. It means that it will be performed for fresh produce mostly during wintertime, when cultivation period for leafy vegetables and soft fruits is ended. In the meantime laboratories have identified the type of fresh produce which will be taken for analysis as well as relevant points of their sales (see Table 22). Representatives from each data gathering laboratory approached managers of shops or retail premises to get permission for the requested fact-finding missions on food safety practices (see Section 3.6). Raspberries as a representative food matrix for soft fruit production chain will be taken for analysis in Finland, Serbia and Poland. Strawberries will be analysed only by the Czech partner.

Table 22. Laboratory/Produce to be analysed in VITAL

Data-gathering laboratory	Fruit	Vegetables	Shellfish	Raw pork products
Czech Republic	Strawberries	-	-	Pork chops and pate
Serbia	Raspberries	Lettuce	-	-
Finland	Raspberries	-	-	-
Greece	-	Lettuce	Mussels	-
United Kingdom	-	-	-	Pork chops and pate
Poland	Raspberries	Lettuce	-	-
Spain	-	-	Mussels	Pork chops and pate
Italy	-	-	-	Pork chops and pate

SOPs revision

The draft SOPs for extraction of enteric viruses from fresh produce, shellfish and meat samples were applied and tested by relevant data gathering laboratories. Any issues that came up were recorded, and all remarks sent to ITACyL. These remarks were taken into account during preparation of the final versions of the SOPs.

Methods implementation

The SOPs for virus extraction and concentration as well as molecular detection of indicator adenoviruses in food samples have been implemented in the data-gathering laboratories. Further work within WP4 regarding sampling and produce analysis will commence according to the agreed sampling schedules.

3.4.1 VITAL WP4 Mid-Term Update (April 1st- October 31st, 2009)

The type of samples which are representative for each food supply chain and points of their sale were finally defined. For soft fruit and vegetables, samples of fresh/frozen raspberries and strawberries, lettuce heads as well as bags of processed vegetables (mixed salads) will be taken. For the pork supply chain there are products containing liver such as pate and pork chops as a raw meat samples. Not all data gathering laboratories have started sampling of the foodstuffs at point of sale, as they are waiting for the fact-finding mission team which will define additional “ad hoc” sampling points. Hitherto a fact-finding mission has been performed in Poland encompassing only selected sites of the point of sale of fruit and vegetables. The fact-finding team has visited wholesale stores as well as farmers’ markets from where samples of fresh produce will be purchased. So far 27 samples of fresh lettuce (T4.2) and 7 samples of fresh raspberries (T.4.4) were taken from farmers’ markets in Poland. Nine samples of frozen raspberries were collected from processing units before shipment to retailer stores (T4.4). Monitoring of the point of sale for fresh fruit was also conducted in the Czech Republic. Samples of strawberries were collected from a supermarket, a farmers’ market and from a farmers’ shop. In total, 38 samples of raspberries allocated for T4.4. and T4.5 were taken. Data-gathering laboratories dealing with monitoring of shellfish and raw pork products will start sample collection after completion of the fact-finding missions related to point of sale.

3.5 Report on Workpackage 5 “Data Analysis” progress and achievements during the period 1st April 2008 to 31st March 2009

Start date or starting event: Month 1

T5.1 A workshop will be held at the 2nd project meeting to train the data gathering partners in the methodology required for the data analysis.

To explain the guidance document on data collection (Deliverable D5.1) and discuss this document for feasibility in particular with the data gathering laboratories, a workshop was organized to be held at the first annual meeting in Serbia from April 6th – April 8th. The preparation of the workshop involved discussions about the most optimal design of the workshop, preparing the agenda and correspondence with partners about their contributions to the workshop.

T5.2 Collect and analyze data for hazard characterization and exposure assessment from WPs 2-4.

This Task does not commence until Month 13.

T5.3 Develop a MPRM for each of the food supply chains for foodborne quantitative virus risk assessment (QVRA).

VITAL involves different food chains, i.e. soft fruits, vegetables, pork and shellfish, and the transmission routes of viruses to humans can differ per chain. Therefore, each of the models describing these routes will require a different chain of modules in the risk models. In the first 12 months of VITAL, the modules for norovirus infection due to oyster consumption have been developed. These modules jointly describe the NoV contamination of oysters from moment of harvest through to moment of consumption. For the moment, data on NoV-contamination that were collected by the RIVM and data from literature were used, but these data will be replaced by VITAL-data once available. The estimated consumed NoV-dose is subsequently related to the dose response model described in literature to characterize the human risk of infection.

A meeting was held on August 6, 2008 using Netviewer to discuss the sample sizes as a first guidance for data collection. This discussion considered the possible benefits and constraints of reducing the sample size by 15% to reallocate budget for site visits by HACCP experts. A reduction of 15% in sample size was concluded to not necessarily negatively affect the quality of data (i.e., estimates for the mean of parameters) in the VITAL setting when the sampling plan remains unaltered, which is the case. What is affected by a reduced number of samples is the uncertainty surrounding the estimated means. A smaller sample size leads to less information about the parameters and thus more uncertainty. Actual effects of the reduction cannot be estimated beforehand, because this is subject to many unpredictable and

uncontrollable circumstances. The unfavourable effects of sample size reduction, however, were compared to the beneficial effects of the site visits. By identifying the control points (and thus sampling points), data collection will be focussed more on the problem areas compared to sampling predetermined points, resulting in more realistic modelling of the food chain. The benefit of this aspect outweighs the increased uncertainty in parameter estimates.

Guidelines for data collection were based on sampling points identified from the background review questionnaires completed as part of WP6. The guidance document on data collection (Deliverable D5.1) was subsequently prepared and circulated to all beneficiaries in March of 2009.

A request for consumption data has been sent out to all beneficiaries in December 2008. These data are essential for assessing *e.g.* the human exposure to viruses in the quantitative virological risk assessment. So far, a response has been received from the Czech Republic, Italy, Poland, Serbia, Slovenia, Spain and UK.

An Excel tool was created for maximum likelihood estimation of the concentration of virus in food and water samples, based on presence/absence data obtained by (RT-) PCR (Deliverable D5.2). The tool is available to all beneficiaries via the VITAL website. The statistical concept of the tool is that of MPN-estimation based on serial 10-fold dilutions. The tool will currently be tested by beneficiaries using real data obtained in the field and the tool will subsequently be improved.

T5.4 To recognize and priorities the main criteria for risk assessment.

Quantitative viral risk assessment and quantitative bacterial risk assessment differ in approach with respect to quantification of pathogen concentration, data needs and dose response models due to the different characteristics of the different pathogen groups. Several of the differences in approach have been recognized during the work performed under Task 5.3. Furthermore, prior conducted risk assessments yielded experience on this matter (See Table 23). The theories about distributions, homogenization, dose-response models and uncertainty and variability that are described in these papers can be valuable for the QVRA performed for VITAL. Furthermore, three papers describe experimental data on water retention on vegetables which may be useful to model the risk of contamination through sprayed irrigation in VITAL. All findings are drafted in a report and placed on the VITAL website (Deliverable D5.3), and will be supplemented throughout the development of the VITAL QVRA.

T5.5 To assess the foodborne virus risks along the developed MPRM in T.5.3 by QVRA .

This Task does not commence until Month 28.

T5.6 To list and if appropriate evaluate effective intervention measures by QVRA

This Task does not commence until Month 25.

Table 23. Quantitative Risk Assessments for Viruses in the Environment

Matrix	Virus	Title publication	Reference
Drinking water	Rotavirus, Poliovirus 1, Poliovirus 3, Echovirus 12	Modelling the risk from <i>Giardia</i> and viruses in drinking water.	Regli et al., 1991
Drinking water	Rotavirus	Risk assessment of virus in drinking water.	Haas et al., 1993
Drinking and recreational waters	Rotavirus	Waterborne rotavirus: a risk assessment.	Gerba et al., 1996
Drinking and recreational waters	Adenovirus	Waterborne adenovirus: a risk assessment.	Crabtree et al., 1997
Salad crops	Enteroviruses	Viral risks associated with wastewater reuse: modeling virus persistence on wastewater irrigated salad crops	Petterson and Ashbolt, 2001
Salad crops	Viruses	Microbial risks from wastewater irrigation of salad crops: a screening - level risk assessment	Petterson et al., 2001
Drinking and recreational waters	Coxsackie virus	Risk assessment of waterborne coxsackie virus.	Mena et al., 2003
Drinking water; Recreational water	Adenovirus	Risk assessment of adenoviruses detected in treated drinking water and recreational water.	Van Heerden et al., 2005
Raw Vegetables	Viruses	Quantitative microbial risk assessment models for consumption of raw vegetables irrigated with reclaimed water	Hamilton et al., 2006
Drinking water	Norovirus	Quantitative risk assessment of noroviruses in drinking water based on qualitative data in Japan	Masago et al., 2006

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3.5.1 VITAL WP5 Mid-Term Update (April 1st- October 31st, 2009)

Task 5.1 *A workshop will be held at the 2nd project meeting to train the data gathering partners in the methodology required for the data analysis.*

A workshop was held during the annual meeting in Novi Sad. The workshop started with an outline of the quantitative virological risk assessment that will be completed within VITAL. The guidance document was subsequently presented to clarify any ambiguities, and to train the data gathering laboratories (DGLs) in collection of the samples. Thereafter, each DGL presented the candidate farms that were selected for sampling. During the subsequent discussions, it became apparent that VITAL would strongly benefit from individual guidance documents for each DGL instead of a general guidance document for all DGLs due to the diversity in production and processing systems between countries. It was concluded that these documents were to be developed within Task 5.2.

Task 5.2 *Collect and analyse data for hazard characterization and exposure assessment from WPs 2-4.*

The general guidance document (Deliverable D5.1) that was sent around in March 2009 was adapted to DGL-specific guidance documents. This process required additional information that could be retrieved partially from the background review questionnaires that were completed within WP6. However, not all background review questionnaires were available at that time. Furthermore, not all required information was present in the returned questionnaire. This information was therefore obtained through the DGLs from the production and processing sites. Location-specific guidance documents were subsequently developed in cooperation with KU Leuven (WP6-leader). In September of 2009, individual guidance documents were sent to the DGLs. For soft fruits, these documents included the final version for the production phase and draft documents for the processing phase for Serbia, Poland and Finland. Furthermore, an individual guidance document for the production phase of soft fruits was sent to the data gathering lab from the Czech Republic. For salad vegetables, individual guidance documents for the production phase were sent to the data gathering labs from Serbia and Poland, while the document for Greece is being developed currently. For the processing phase of salad vegetables, draft guidance documents were sent to the data gathering labs from Serbia and Greece. Completion of all draft guidance documents is pending on further information that is to be obtained from the different locations. The draft guidance documents will be finalized by November of 2009.

Task 5.3 *Develop a MPRM for each of the food supply chains for foodborne quantitative virus risk assessment (QVRA).*

The Modular Process Risk Model (MPRM) was further elaborated by identifying mathematically nine modules for the soft fruit production chain: virus attachment and detachment from soft fruit due to irrigation, virus attachment to harvester's hands, virus transfer from harvester's hands to product, viral contamination of soft fruits by animal manure, viral contamination of soft fruits due to washing, viral contamination of soft fruits due to contact with equipment, contamination of soft fruits due to food handler's hands, consumption of soft fruits and natural inactivation of the virus. The modules for salad vegetables and pork products will be identified next. Part of these modules will be the same as those identified for the soft fruits production chain.

Task 5.4 *To recognize and prioritise the main criteria for risk assessment.*

Between April 2009 and October 31st 2009, no new papers on quantitative virological risk assessment were published that could aid the theoretical framework of the VITAL QVRA. Therefore, Deliverable D5.3 could not be updated during the third half-year of VITAL. However, the identification of the nine modules as described under Task 5.3 showed the data needs for the QVRA with respect to soft fruits processing and production. These needs were used to identify the sampling points as described in the guidance documents. Because it will not be feasible to obtain all the required data in the field, the data needs will be fulfilled additionally by the PhD studies that are conducted within VITAL as part of WP6.

T5.5 *To assess the foodborne virus risks along the developed MPRM in T5.3 by QVRA.*

This Task does not commence until Month 28.

T5.6 *To list and if appropriate evaluate effective intervention measures by QVRA.*

This Task does not commence until Month 25.

3.6 Report on Workpackage 6 “Control Measures” progress report 1st April 2008 to 31st March 2009

Start date or starting event: Month 1

T6.1 Critical evaluation of current HACCP systems.

Objective

To review information on current monitoring and control practices used in the food industry against infectious agents.

To assess foodborne viral risks for determining high risk situations and the efficacy of interventions.

Results

Data gathering on current HACCP and other food safety systems using two approaches: 1. a bench study of information available on the internet and in scientific literature; 2. background review questionnaires and visits to food businesses within KULeuven’s network of partners. Both generic and specific information was obtained on food process flows charts, food safety systems in place, assessment of CCP’s, prerequisite conditions, etc.

During the first 6 months it was decided by the RDMB to strongly link the fact-finding missions on food safety practises to the sampling of the different sites/points in order to ensure that:

- the sampling points are realistic to the actual production situation.
- at the moment of sampling the actual standards of both operational and structural hygiene are documented and linked to the sampling results and subsequently will be used as a basis for the development of a Code of Practice.

It is essential for the outcome of VITAL to perform full HACCP fact-finding missions for the production and processing phases, which will encompass the fact-finding missions on food safety practises to the farms and factories from which the food samples will be taken in WPs 2-4. The fact-finding process consists of the following steps:

Completion of three background review questionnaires per step in the supply chain by each partners participating in the sampling.

- Evaluation of the completed background review questionnaires in order to select the initial sampling sites/points.
- Formulation of a fact-finding plan taking into account sampling schedules, seasonal aspects of processes and budgetary constraints.
- Carrying out the fact-finding missions on food safety practises. The fact-finding team will visit the site, identify the points to be sampled, and samples will be taken by members of the data-gathering laboratory.
- Analysis of the findings and completion of the fact-finding reports.

Catholic University of Leuven (KULeuven) – In fulfilment of its tasks as WP Leader KULeuven has achieved the following results:

Data gathering on current HACCP and other food safety systems. At present the following information is available

Bench study - Generic information on the food supply chains of vegetables, fruits and pork meat.

Visits to food businesses –Detailed information on actual practices in the food supply chain of vegetables which are manufactured in their raw state for processing and/or consumption by the customer based on visits within KULeuven’s network of partners.

Finalisation of an collaborative agreement between KULeuven and the HSE Ireland in order to utilise auditing experience of a HSE Environmental Officer, i.e. Rita Moloney. This person has a 35 years of auditing experience, advanced HACCP training and is certified Lead Quality Auditor of ISO. This collaborative agreement came about as a result of previous collaborative work with KULeuven on hand hygiene in the food industry.

Coordination and organisation of fact-finding process/plan.

Background review questionnaires – Background review questionnaires were finalised and sent out to the participating partners, i.e. producers fruit, vegetables, pork; processing fruit and vegetables.

Evaluation of background review questionnaires – The submitted background review questionnaires were analysed and reported at the 1st annual meeting. Sampling points for producers fruit and vegetables are defined in collaboration with RIVM but need further fine-tuning.

Fact-finding mission plan – Most participants have submitted their sampling schemes and the finalisation of the fact-finding missions plan is in progress. The fact-finding missions on food safety practises are expected to commence in June 2009.

University of Ljubljana – As fulfillment of its task UL-BF has achieved the following results:

Preparation of the background review questionnaires in collaboration with KULeuven.

Mateja Ambrožič attended the course Verification of food safety management system with internal audit of HACCP system and ISO 22000:2005 by Slovenian Institute of Quality and Metrology (SIQ).

Two review articles are being prepared, one on the methods for detection of foodborne viruses and the other on existing codes of good practice and their impact on food borne viruses.

T6.2 Survival and elimination of viruses.

Objective

To develop new measures to prevent virus contamination of foods and the environment.

Results

University of Barcelona (UB) – As expert laboratory in adenoviruses and viruses used for microbial source tracking, UB has in fulfillment of its tasks selected and recruited a PhD student to carry out the planned work activities. The following results have been achieved:

- Training of the PhD student in the laboratory for techniques commonly used in the study of food-borne viruses, i.e. culturing, concentrating, detecting and quantifying viruses
- Stocks of adenoviruses have been produced and worked has been carried out on the quantification by qPCR and infectivity assays of human adenoviruses. The work has been done for Murine norovirus, cultured in RAW cell line, which will be used as process control between laboratories.
- Survival and disinfection studies have been initiated with the development of experimental protocols for chlorine disinfection in a diversity of types of water and initial data on disinfection for adenoviruses has been produced.
- DNA standards have been prepared by linking the target sequence to a plasmid and preparing serial dilutions in order to quantify the viral load decay by an optimized qPCR methodology.

UB has also participated in the pre-collaborative ring trials 1 and 2 for adenoviruses and noroviruses for both raspberries and pork meat and in an external ring trial for molecular detection and quantification of noroviruses.

Following the tasks assigned to the UB laboratory as laboratory expert in adenoviruses and viruses used as microbial source tracking, the lab of the UB has also been training for 2 weeks a member of ITACyL group and tested samples collected by this group for porcine adenoviruses by qPCR and nested-PCR tests.

Wageningen University Research (WUR) / Defra

National Institute for Public Health and the Environment (RIVM) – In fulfilment of its task RIVM has selected and recruited a PhD student, i.e. Katharina Verhaelen, to carry out the planned work activities. The following results have been achieved between January – April 2009:

- Training of the PhD student in the laboratory for techniques commonly used in the study of food-borne viruses, i.e. culturing, concentrating, detecting and quantifying viruses.
- Pre-collaborative ring-trials.
- Quality plan and detailed project proposal for PhD research.
- Thorough literature study focusing on known, novel and validated processes for norovirus inactivation in food products especially fresh produce.

T6.3 Development of HACCP models.

Objective

To develop and assess measures for virus reduction and control in case of virus contamination.
Develop HACCP models for each supply chain

Results

This task integrates the information acquired from tasks 6.1 and 6.2 with the MPRMs developed in task 5.2 using data gathered in WPs 2 and 3. This Task will commence full development as soon as the data-gathering proceeds in Summer 2009.

T6.4 Evaluation of the effect of vaccination.

Objective

To develop and assess means for virus reduction and control in case of virus contamination.
Evaluate the efficacy of swine vaccination strategies against HEV to decrease virus transmission and to reduce the risk of contaminated meat.

Results

Wageningen University Research (WUR) – In fulfilment of its task WUR has chosen the following strategy:

- to model HEV outbreaks in pigs at farm level using existing experimental data
- to evaluate and refine the model using field data
- to extend the model with an intervention strategy (vaccination) and assess the efficacy of vaccination using different scenarios.

During the first year the following results have been achieved:

- Development of HEV outbreak model. A SEIR (susceptible- exposed- infectious- recovered) model was used, and experimental data were obtained from Martijn Bouwknegt et al. 2008. (Estimation of hepatitis E virus transmission among pigs due to contact exposure. *Vet. Res.* **39** 40). In the experiments a high reproduction number ($R_0 = 17$) was found, and using this estimation in the model it was concluded that all infections take place during the first part of the fattening period. This implies that at slaughter age no HEV excreting pigs are present, and the risk of contamination of the slaughter line or meat products is minimal. However, if transmission is lower (e.g. $R_0 = 2.6$), the number of pigs excreting at slaughter age is around 10%, which poses a threat of infection of the slaughter line and meat products. It may be concluded that the reproduction number determines the number of virus-excreting pigs at slaughter age. Since we only have experimental data to use, and since it is not clear whether these data are representative for the field situation, the next step would be to use field data in the model. The data gathered from farms (see Table 6) will be taken with the aim to use these for the vaccination modelling study.

3.6.1 VITAL WP6 Mid-Term Update (April 1st- October 31st, 2009)

T6.1 Critical evaluation of current HACCP systems

T6.1 Objective

To review information on current monitoring and control practices used in the food industry against infectious agents.

To assess food borne viral risks for determining high risk situations and the efficacy of interventions.

T6.1 Results

Data gathering on current HACCP and other food safety systems using two approaches: 1. A bench study of information available on the internet and in scientific literature; 2. Background review questionnaires and visits to food businesses within K.U.Leuven's network of partners. Both generic and specific information was obtained on food process flow charts, food safety systems in place, assessment of CCP's, prerequisite conditions, etc.

During the first 6 months it was decided by the RDMB to strongly link the fact-finding missions on food safety practices to the sampling of the different site/points in order to ensure that:

- The sampling points are realistic to the actual production situation
- At the moment of sampling the actual standards of both operational and structural hygiene are documented and linked to the sampling results and subsequently will be used as a basis for the development of a Code of Practice

It is essential for the outcome of VITAL to perform full HACCP "fact-finding" for the production and the processing phases, which will encompass fact-finding missions on food safety practices to the farms and factories from which the food samples will be taken in WPs 2-4. The fact finding process consists of the following steps:

- Completion of three background review questionnaires per step in the supply chain by each partner participating in the sampling.
- Evaluation of the completed background review questionnaires in order to select the initial sampling site/points.
- Formulation of a fact-finding plan taking into account sampling schedules, seasonal aspects of processes and budgetary constraints.
- Carrying out the fact-finding mission on food safety practices. The fact-finding team will visit the site, identify the points to be sampled, and samples will be taken by members of the data-gathering laboratory.

- Analysis of the findings and completion of the fact-finding reports.

Catholic University of Leuven (K.U.Leuven) - In fulfillment of its tasks as WP Leader K.U.Leuven has achieved the following results:

- Data gathering on current HACCP and other food safety systems – Currently the following information is available:
 - o Bench study – Generic information on the food supply chains of vegetables, fruits and pork meat.
 - o Visits to food businesses – Detailed information on actual practices in the food supply chain of vegetables which are manufactured in their raw state for processing and/or consumption by the customer based on visits within the K.U.Leuven’s network of partners. Additionally, similar information is available on the food supply chain of pork meat, i.e. from slaughterhouse to retail.
- Collaborative agreement between K.U.Leuven and HSE – K.U.Leuven (Belgium) signed a collaborative agreement with the Health Safety Executive (HSE – Ireland) in order to utilize the auditing experience of a HSE Environmental Officer, i.e. Rita Moloney. This person has a 35 years of auditing experience, advanced HACCP training and is certified Lead Quality Fact-finding Auditor of ISO. This collaborative agreement came about as a result of previous collaborative work with K.U.Leuven on hand hygiene in the food industry.
- Organisation and carrying out fact-finding missions – Most participants have submitted their sampling schemes and the finalisation of the fact-finding plan is on-going. The fact-finding missions on food safety practices are in progress. The following results have been achieved:
 - o Compilation of background review questionnaires – For each stage of the relevant food supply chains the following background review questionnaires have to be compiled, i.e.: 1. fruit & vegetables: primary production, processing, point-of-sale; 2. pork meat: slaughter house, meat processing and point-of-sale.

These background review questionnaires are based on best practice and EU legislation. Non compliance is based solely on the EU legislation i.e. is the minimum legislative requirement.

Each questionnaire consists of 5 modules namely; (1) enterprise (farm) review, (2) quality management systems, (3) physical location and lay-out, (4) production process, (5) product quality and traceability. All sections of the modules may not be relevant to each food business operation.

All of the background review questionnaires have been developed with the exception of the pork retail one which is currently being finalised.

- o Completion of the background review questionnaires – Each participating country to complete 3 background review questionnaires for each stage of the relevant food chain. These completed

background review questionnaires provide invaluable data and also ensure that there is variety regarding the final selection

When selecting the food businesses the data gathering laboratories must ensure that each stage of the supply chain is directly linked.

Table 24 Current status of the background review questionnaires (date: 27.11.09)

	PRODUCTION	PROCESSING
FRUIT	CZ: 3	CZ: 1
	FI: 1	FI: 1
	PL: 4	PL: 1
	RS: 4	RS: 1
VEGETABLES	GR: 2	GR: 2
	PL: 2	PL: 0
	RS: 4	RS: 1
PORK MEAT	CZ: 1	CZ: 2
	IT: 0	IT: 1
	SP: 1	SP: 1
	UK: 1	UK: 0

- *Selection of the food businesses* – The completed background review questionnaires are analysed and a final selection is made.

Areas of concern and possible sampling points are highlighted and discussed with RIVM in order to support the development of guidance documents for sampling.

- *Preparation for fact-finding mission* – A checklist is prepared using the information furnished by the relevant questionnaire.

Liaison with the relevant data gathering laboratories and logistic arrangements for the fact-finding mission are finalised.

- *Completion of fact-finding missions* – The mission is carried out in the company of the local representative and sampling team of the data gathering laboratory. General samples and ad hoc samples identified during the course of the visit are taken.

Table 25 Current status of fact-finding missions (date: 27/11/09)

	PRODUCTION	PROCESSING
FRUIT	CZ: 0	CZ: 0
	FI: 1	FI: 1
	PL: 1	PL: 1
	RS: 2	RS: 1
VEGETABLES	GR: 1*	GR: 1*
	PL: 1	PL: 0
	RS: 1	RS: 0
PORK MEAT	CZ: 0	CZ: 0
	IT: 0	IT: 0

	SP: 0	SP: 0
	UK: 1	UK: 0

* FFM planned on 07-10/12/09

- o Completion of reports – The report includes verified information originally provided in the background review questionnaires, areas of concern noted during the fact-finding mission with photographic evidence where relevant and appropriate and a list of samples taken. Prior to publication a draft of the report is sent to the data gathering laboratory for verification.

Table 26 Current status on FFM reports (date 27/11/09)

	PRODUCTION	PROCESSING
FRUIT	CZ: 0	CZ: 0
	FI: 1	FI: v
	PL: v	PL: 1
	RS: v	RS: 0
VEGETABLES	GR: 0	GR: 0
	PL: 1	PL: 0
	RS: v	RS: 0
PORK MEAT	CZ: 0	CZ: 0
	IT: 0	IT: 0
	SP: 0	SP: 0
	UK: 0	UK: 0

“0”: not completed; “1”: completed; “v”: in preparation

T6.1 Problems

- Non completion of the required number of background review questionnaires. This limits our choice regarding the best company which could fulfill VITAL’s objectives.
- Background review questionnaires not completed within the allocated time frame. This presents a logistical nightmare regarding scheduling of our own work commitments and other fact finding missions. We had hoped that we could focus on completing the outstanding processing fact finding missions and all of the retail ones in early 2010. However we still have to complete some of the outstanding primary production missions. Last minute scheduling of missions has also proved costly regarding flights and accommodation

T6.1 Acknowledgements

A special thanks to all of the data gathering laboratories who have completed and are in the process of completing background review questionnaires. It is acknowledged that this can be a very laborious and timely process and that in some cases great problems are encountered in securing cooperation with different companies. Also we wish to acknowledge the wonderful hospitality and assistance received during the fact finding missions.

T6.2 Survival and elimination of viruses

T6.2 Objective

To develop new measures to prevent virus contamination of foods and the environment by studying the survival and elimination of viruses.

T6.2 Results

University of Barcelona (UB) – The team at the University of Barcelona, is involved in Task .6.2 as an expert laboratory in adenoviruses and viruses used for microbial source tracking. UB has in fulfillment of its tasks selected and recruited a PhD student to carry out the planned work activities. The following results have been achieved:

- Training of the PhD student – The PhD student was trained in the laboratory for techniques commonly used in the study of food-borne viruses, i.e. culturing, concentrating, detecting and quantifying viruses.

In addition UB has also trained a member of ITACyL group for 2 weeks and tested samples collected by this group for porcine adenoviruses by qPCR and nested-PCR tests.

- Preparative activities - Preparation of cell cultures, viral strains and DNA standards for both human adenoviruses and murine noroviruses.
- Protocol development for infectivity assays – Different types of infectivity assays were compared for Murine norovirus and adenoviral strains 2 and 41, including Plaque Forming Units (PFU), TCID50 and immunofluorescence assays using different available antibodies. This resulted in the selection of the following final protocols:
 - o Protocol for quantification of virus stability by qPCR and by infectivity assays;
 - o Plaque forming units (PFU) counting protocols for human adenovirus 2 using A549 cells and murine norovirus using RAW 264.7 cells;
 - o Protocol for immune-fluorescent assays for Human adenovirus 41 using 293 cells.
- Linking qPCR results with infectivity results - Enzymatic treatments with proteinase and DNase/RNase have been applied to the experiments in order to link the quantification results based on number of genome copies obtained by qPCR with the infectivity results, avoiding quantification of potentially free viral nucleic acid by the qPCR assays.
- Protocol development for disinfection studies - Development of experimental protocols for chlorine disinfection in a diversity of water types. Development of a protocol for the preparation of viral suspensions to be used in the study of UV disinfection processes in adenoviruses. This included the

evaluation of diverse methods for the dispersion of viral particles potentially aggregated in the suspensions.

- Data gathering on survival and elimination - Initial data on disinfection efficiency of chlorine for human adenovirus 2 and murine norovirus in chlorine demand free buffer, river water, seawater and artificial seawater and preliminary information of inactivation kinetics analyzing the viruses by qPCR and infectivity assays have been produced.
- (Pre)validation of test protocols - UB has participated in the pre-collaborative ring trials 1, 2, 3 and 4 as well as the external ring trials for the molecular detection and quantification of adenoviruses and noroviruses in both raspberries and pork meat.

Wageningen University Research (WUR) / VLA – In fulfillment of its task WUR/VLA has selected and recruited a PhD student, i.e. Alessandra Berto, to carry out the planned work activities in Task 6.2. The focus is on obtaining an optimized culture propagation method for HEV in order to be able to demonstrate the infectivity of any virus detected, which is central to the significance of PCR detection of zoonotic viruses in the food chain. The following results have been achieved:

- Training of the PhD student – The PhD student was trained in the laboratory for techniques commonly used in the study of food-borne viruses, i.e. culturing, concentrating, detecting and quantifying viruses.
- Monolayer cultures - Monolayer cultures of HepG2/C3A and PLC/PRF/5 cells were inoculated with HEV extracted from PCR-positive fecal suspensions. Despite several attempts using different fecal samples, no increase in HEV RNA copy number was observed in any cultures, using quantitative real-time RT-PCR.
- 3D cell cultures - 3D cultures of the same cell lines were prepared and infected with HEV extracted from the liver of an experimentally infected pig (obtained from WUR). Non infected cultures were set up in parallel. As further controls, 2D cultures were set up and inoculated as the 3D cultures.

Increases in HEV RNA copy number were observed beginning at 14dpi and continued to increase for more than 60 days in culture. No increases in HEV RNA copy number were observed in any of the 2D cultures. These results were further confirmed by IPX staining of infected cultures using a HEV monoclonal antibody obtained from ISS.

Based on these results the 3D culture technique represents an excellent result and a breakthrough for work package 6.2. However, as operated, the 3D system is not suitable for examining large numbers of samples for HEV.

- Evaluation of higher throughput - In order to better evaluate the facility of the system for examination of higher sample numbers, a trial to compare the infection efficiency of (1) 3D vs (2) 2D vs (3) 3D transferred to 2D just prior to inoculation with sample was set up. The hypothesis was that the greater permissivity of cells established by conditioning and differentiation in the 3D system would be retained for some time after the cells were transferred to a 2D system, enabling multi-well plates to be set up for examination of high sample numbers.

The 3D transferred to 2D gave inconsistent results but overall indicated that the progeny of the 3D *in-vitro* propagation was infective. The inconsistency was probably a result of the transfer of differing numbers of cells with the supernatant. In addition these studies showed that in cells grown in 3D and then transferred to 2D for infection, HEV propagation was more efficient than 2D. Further refinements are being implemented to this system and these experiments are in progress. If successful this should enable screening of large numbers of pig and environmental samples for the presence of viable HEV, and thereby the establishment of their significance in autochthonous, zoonotic transmission.

- Construction of an interferon knockout cell line - A further experiment has begun to construct an interferon knockout cell line, with the aim of improving sensitivity (i.e. HEV permissivity) of *in-vitro* detection system.
- (Pre)validation of test protocols – WUR/VLA has participated in the pre-collaborative ring trials 1, 2, 3 and 4 as well as the external ring trials.

In September 2009 feces and liver samples were collected in an UK abattoir within the VITAL WP2. These samples will be tested by PCR for HEV detection and any positives identified will be used as inocula for culture systems (1), (2) and (3) both wild type and IFN-KO. This will give us more information about the relative sensitivity of the *in-vitro* methods and by extension about the infectivity of HEV detected in the food chain, and also link data and expertise obtained within different VITAL work packages.

National Institute for Public Health and the Environment (RIVM) – In fulfillment of its task RIVM has selected and recruited a PhD student, i.e./ Katharina Verhaelen, to carry out the planned work activities in Task 6.2. Detection of human norovirus on foods is hampered by the lack of a cell culture system. Though in 2007 Straub et al. have published a 3-dimensional, organoid model of human small intestinal epithelium, this could not be reproduced by them or any other research group. A recent murine norovirus variant was found to be very promising surrogate for human norovirus on foods which will be used in further persistence and inactivation studies. Therefore, cell culture and PCR of MNV-1 will be compared with detection of human norovirus by PCR with/without pretreatment and/or after transfection. The following results have been achieved:

- Training of the PhD student – The PhD student was trained in the laboratory for techniques commonly used in the study of food-borne viruses, i.e. culturing, concentrating, detecting and quantifying viruses.
- Preparative activities - Quality plan and detailed project proposal for PhD student. Thorough literature study focusing on known, novel and validated processes for norovirus inactivation in food products especially fresh produce.
Preparation of RAW-264.7 cell cultures used for MNV-1 propagation, viral strains and DNA standards for murine noroviruses.
- Protocol development for infectivity assays – A plaque assay was tested to quantify infectious virus particles. This resulted in a final Plaque forming units (PFU) counting protocol for murine norovirus using RAW 264.7 cells
- (Pre)validation of test protocols – RIVM has participated in the pre-collaborative ring trials as well as the external ring trials.

Approaches aiming to distinguish between infectious and defective hNoV particles in nRT-PCR, such as an enzyme treatment, will be tested later with actual samples of experiments, e.g. after high pressure treatment.

T6.3 Development of HACCP models

T6.3 Objective

To develop and assess measures for virus reduction and control in case of virus contamination.

Develop HACCP models for each supply chain.

T6.3 Results

This task integrates the information acquired from tasks 6.1 and 6.2 with the MPRMs developed in task 5.2 using data gathered in WPs 2 and 3. This task will commence full development as soon as the data-gathering is finalised during 2009 and 2010.

However, the development of the CA COP has made an independent VITAL COP redundant, and therefore some changes to VITAL are necessary to ensure that the work of VITAL would retain assured full relevance for food safety management. The relevance and impact of VITAL's work would furthermore be enhanced by its incorporation in some form into the CA COP. It was agreed that the main impact of the development CA COP would be on VITAL's Tasks 6.3 "Development of HACCP models" and Task 7.1 "Development and validation of a Code of Good Practice".

Task 6.3 would be entitled “*Translation of Codex Alimentarius Code of Good Practice for virus management to HACCP models for specific food supply chains*”. HACCP models, directed towards the control of virus contamination, would be developed for each food supply chain, by evaluating the information acquired from T6.1 and T6.2, then integrating the MPRMs developed in T5.2 using data gathered in WPs 2, 3, and 4. Guidance manuals will be produced (containing) the HACCP models) as an aid to virus-relevant HACCP implementation in specific supply chains. These documents will integrate all Codex principles and will translate them to practical solutions for the particular food supply chains. The deliverable from this Task will be Guidance manuals for the salad vegetable, soft fruit and pork supply chains, containing HACCP models for managing food-borne virus contamination in these supply chains.

T6.4 Evaluation of the effect of vaccination

T6.4 Objective

To develop and assess means for virus reduction and control in case of virus transmission and to reduce the risk of contaminated meat.

T6.4 Results

Wageningen University Research (WUR) – In fulfilment of its task WUR has chosen the following strategy:

- To model HEV outbreaks in pigs at farm level using existing experimental data.
- To evaluate and refine the model using field data.
- To extend the model with an intervention strategy (vaccination) and assess the efficacy of vaccination using different scenarios.

At present the following results have been achieved:

Development of HEV outbreak model - A SEIR (susceptible- exposed- infectious- recovered) model was used, and experimental data were obtained from Martijn Bouwknegt et al. 2008. (Estimation of hepatitis E virus transmission among pigs due to contact exposure. Vet. Res. 39: 40). In the experiments a high reproductive number ($R_0 = 17$) was found, and using this estimation in the model it was concluded that all infections take place during the first part of the fattening period. This implies that at slaughter age no HEV excreting pigs are present, and the risk of contamination of the slaughter line or meat products is minimal. However, if transmission is lower (e.g. $R_0 = 2.6$), the number of pigs excreting at slaughter age is around 10% which poses a threat of infection of the slaughter line and meat products. It may be concluded that the reproduction number determines the number of virus-excreting pigs at slaughter age. Since we only have experimental data to use, and since it is not clear whether these data are representative for the field situation, the next step would be to use field data in the model. The data

gathered from farms will be taken with the aim to use these for the vaccination modelling study. Sampling will start in February/march 2010.

3.7 Report on Workpackage 7 “Delivering Impact” progress and achievements during the period 1st April 2008 to 31st March 2009

Start date or starting event: Month 1

T7.1 Development and validation of a Code of Good Practice

This Task commenced in Month 10. Abstracts for analysis were selected at on-line databases on the fields “Food Science and Technology Abstracts” and “PubMed”. The main keyword to select the appropriate journal articles was “food safety” and the years of interest were from 1969 to 2008. Selection was based on content analysis of abstracts and determination of keywords from the text. Afterwards, keywords were integrated into 8 groups (hygiene, viruses, epidemiology, consumer, legislation, regulation, inspection, education/training) and several subgroups. It is very apparent that viruses as hazards in food supply chains are poorly recognized, which underpins the need for a code of good practice for foodborne viruses.

T7.2 Symposium organization

This Task does not commence until Month 25, however a preliminary programme for the Symposium “New Developments in Monitoring and Control of Foodborne Viruses”, which will be held in Ljubljana in 2011, was prepared and disseminated to VITAL participants.

T7.3 Workshop organization

This Task does not commence until Month 25, however discussions have commenced regarding the workshop, its scope and possible participants and invited persons. The discussions will be continued through to the 3rd Consortium Meeting, when the final plan will be fixed upon.

T7.4 Organization of a 1 day training course on foodborne viruses for risk managers in the food industry

As for T7.3 above.

T7.5 Website construction and maintenance

A project website was produced in the first month. A domain name of www.eurovital.org was purchased for a minimum of three years. The website was created and is maintained by FERA, (Defra, UK) using the Adobe® Contribute® program. There are two main sections which make up the website. The homepage shows all the publicly accessible information pertaining to the project, including “Public deliverables”, “Women in Science”, “Presentations” and “Participating Institutes”.

There is also a dedicated section for both the beneficiaries and the expert stakeholders, which are both secure access, and require a username and password to gain entry. Currently, the expert stakeholders section does not contain any information additional to the “Beneficiaries” section. The expert stakeholders also have access to the beneficiaries section. Within the beneficiaries section can be found comprehensive details including the minutes of the RDMB, the PAB and the CAT meetings. There are also sections dedicated to the SOPs for the project and contact details for all the participants (“Contacts”). On the main beneficiaries page there can also be found the grant agreement, the DoW, and details of the MTAs for the project.

The website is updated regularly (approximately once per week).

T7.6 Consortium Meeting Organisation.

During the first project year, two meetings were organized and one held.

The kick off meeting was organized at the Veterinary Research Institute (VRI) in Brno, Czech Republic between 7th and 8th April 2008. Fifty-five people participated (including 23 VRI employees). As part of the meeting there was an organised visit to a dairy farm (BONAGRO s.r.o, Blažovice), where meeting participants observed the complete process of dairy production from cattle husbandry and manure management through to bulk milk handling.

The Scientific Veterinary Institute “Novi Sad”, in Novi Sad, Serbia, organized during the latter months of the first project year the 2nd Consortium Meeting, to be held in the Hotel Park in Novi Sad. A visit to a fruit production site (Libertas d.o.o, Sabac) has been organized as part of the meeting.

3.7.1 VITAL WP7 Mid-Term Update (April 1st- October 31st, 2009)

In this period, a study has been performed in order to analyze terms connected to “food safety” in the period from 1969 to 2008 in major on-line databases for the field, such as “PubMed” and “Food Science and Technology Abstracts” (FSTA) using content-analysis as a methodological tool. Main results revealed inconsistent use of “food safety” terminology in the food safety field. It has been shown that professionals in the field do not use the same terminology with totally comparable meaning of relevant terms concerning food safety issues. With regard to global food safety in food supply chain this paper will point out the need for uniformed terminology, because inconsistent terminology is deceptive and can be one of the risk factors at professional and scientific level.

T.7.1. Development and validation of a Code of Good Practice.

After the revised tasks for 7.1, we are now dealing with task 7.1a Systems verification of the Codex Alimentarius COGP for control of viruses in foods. In October we started carrying out the SWOT analysis of relevant COGPs around the globe. This will give us within upcoming months a critical comparison with the CA draft.

T.7.2. Symposium organisation. The Code of Good Practice will be distributed to interested parties from the food industry at the symposium and workshop.

T.7.3. Workshop organisation.

T.7.4 Organisation of two 1-day training courses on foodborne viruses for risk managers in the food industry

An agenda for the *symposium* has been proposed by the University of Ljubljana (who will be the organiser) and then was sent to all beneficiaries and discussed in RDMB in July and October. The congress will be held in Ljubljana and it will last 3 days in March 2011 (from 14-17 March 2011), but with proposed new amendments, the congress can be conducted in June 2011, what would enable higher visibility of results after all project activities will be finished. Also the organising committee have been proposed and it is under discussion to be organised. Also initial planning of the workshop and training courses (schedule, aim, duration, participants etc.) have been done between the WP leader and the Organising committee of all the events.

T.7.5 Web-site construction and maintenance.

The project website is active and fully informative with all the update information about the progress of the project.

T.7.6 Consortium Meeting Organisation. Two VITAL Consortium Meetings have been organised so far. The first meeting, in Month 1, has taken place at the Veterinary Research Institute, Brno, Czech Republic (VRI) and the Second VITAL Consortium Meeting has taken place at the Scientific Veterinary Institute “Novi Sad”, Serbia (NIV-NS). 6 RDMB meetings have been held up till October 31st 2009.

4. Deliverables and milestones tables

Deliverables (excluding the periodic and final reports)

TABLE 27. DELIVERABLES³

Del. no.	Deliverable name	WP no.	Lead beneficiary	Nature	Dissemination level	Delivery date from Annex I (proj month)	Delivered Yes/No	Actual / Forecast delivery date	Comments
D7.1	Project web-site	7	UPA	O	PU	1	Yes	1	
D1.1	Minutes of 1st PAB meeting	1	Defra	R	RE	1	Yes	1	
D1.2	Minutes of 1st CAT meeting	1	Defra	R	RE	2	Yes	2	
D1.3	Minutes of 1st RDMB meeting	1	Defra	R	RE	3	Yes	3	
D1.4	Minutes of 2nd CAT meeting	1	Defra	R	RE	4	Yes	4	
D1.5	Minutes of 2nd RDMB meeting	1	Defra	R	RE	6	Yes	7	
D1.6	Minutes of 3rd CAT meeting	1	Defra	R	RE	6	Yes	6	
D5.2	A tool for data analysis	5	RIVM	R	RE	8	Yes	9	
D1.7	Minutes of 4th CAT meeting	1	Defra	R	RE	8	Yes	8	
D1.8	Minutes of 3rd RDMB meeting	1	Defra	R	RE	10	Yes	10	
D1.9	Minutes of 5th CAT meeting	1	Defra	R	RE	10	Yes	11	
D5.1	A guidance document on data collection and analysis	5	RIVM	R	RE	12	No	13	
D5.3	Document on available models	5	RIVM	R	RE	12	No	13	
D1.10	Minutes of 6th CAT meeting	1	Defra	R	RE	12	No	13	

Milestones

TABLE 28. MILESTONES							
Milestone no.	Milestone name	Work package no	Lead beneficiary	Delivery date from Annex I	Achieved Yes/No	Actual / Forecast achievement date	Comments
M2.1	All data-gathering laboratories fully prepared with necessary materials and SOPs	2	WUR	9	Yes	10	

5. Project management

Project Management in VITAL is performed within **Work Package 1 “Management”**. WP1 is divided into 2 tasks, brief summaries of the roles of which within Year 1 follow:

Task 1.1 Project administration

The Core Administration Team (CAT), composed of the Coordinator, Assistant Coordinator, Financial Coordinator and Project Legal Officer, has performed the maintenance of the consortium agreement, and the overall legal and administrative management of the project. In this first project year, the CAT was primarily involved with the finalization of the project Grant Agreement, the finalization of the Consortium Agreement, and the negotiation and finalization of Material Transfer Agreements with various institutes. The CAT met formally on six occasions within the first project year, on the 7th May 2008, 8th July 2008, 11th September 2008, 11th November 2008, 16th January 2009, and the 17th March 2009. All CAT meetings were held at the Central Science Laboratory (now the Food and Environment Research Agency). The minutes of these meetings were project Deliverables, and a copy of each is provided in Annex 1 of this Report.

Task 1.2 Overseeing project progress.

A key responsibility of the CAT within T1.2 is to liaise with the Research and Dissemination Management Board (RDMB), assisting this body to monitor the milestone progress towards the achievement of VITAL’s objectives, identifying any causes of non-delivery and taking appropriate corrective action if necessary. The RDMB met formally on three occasions within the first project year, on the 17th June 2008, 16th September 2008, and the 21st January 2009. All these meetings were conducted using a net-based conferencing facility (Netviewer UK Ltd, Guildford, UK). The minutes of these meetings were project Deliverables, and a copy of each is provided in Annex 1 of this Report.

A second key responsibility of the CAT within T1.2 CAT members is to liaise with the Project Advisory Board (PAB), ensuring that advice from this body is implemented within the project. A

meeting was held during the 1st Consortium Meeting on April 7th 2008 at the Veterinary Research Institute, Brno, Czech Republic. The minutes of this meeting was a project Deliverable, and a copy is provided in Annex 1 of this Report.

Specific management details from the 1st reporting year

Problems which have occurred and how they were solved or envisaged solutions

The University of Barcelona has requested that MTAs are signed before the SOPs for the QPCR assays for porcine adenovirus and bovine polyomavirus are released to the Consortium. A draft MTA was drawn up, to be signed by all beneficiaries. However, some beneficiary institutes would not agree to signing this MTA, as it was under Spanish law and they preferred to sign under their own country's laws or those of Belgium. However, UB indicated that they were willing to enter into individual Agreements with these beneficiaries, and finally MTAs were signed to everyone's satisfaction.

A similar situation was encountered regarding the identification of a suitable process control virus in Task 2.1. It was initially envisaged that VITAL could use the virus suggested by the TAG4 group. However, this virus, a mengovirus, is in fact genetically modified and requires a licence for handling, and some beneficiaries considered that it would be difficult to obtain this licence. Therefore an alternative virus was considered, and after extensive consultation and communication among the consortium it was decided to use the recently discovered murine norovirus, as it is morphologically identical to human norovirus. Murine norovirus could be obtained from Washington University in the United States; however, they requested that the Consortium signed an MTA before they will allow us to use the virus. Again, all beneficiaries have signed the MTA save KULeuven and RIVM.

It became clear in the early months of the project during the full discussions amongst the VITAL consortium, and especially the leaders of WPs 6 and 7, that it is essential for the outcome of VITAL to perform full HACCP fact-finding missions for the production and processing phases, which will take the form of the fact-finding missions on food safety practises to the farms and factories from

which the food samples will be taken in WPs 2-4. This is essential so that the sampling points chosen will reflect any risk of virus contamination and embody the points at which contamination may be controlled, and to fully inform the Code of Good Practice, as it will be drawn up following an accepted standard fact-finding missioning procedure. In consequence, this would truly fulfil the aim of the project to integrate monitoring and control of foodborne viruses within food supply chains.

Because the fact-finding missions on food safety practises would now be fully integrated with the sampling plans, the visits would be more extensive and frequent. We therefore wished to allocate 40,000 euros for the fact-finding missions. We had 12,500 euros for Task T6.1 already allocated, but needed to redistribute the extra 27,500 euros from other areas within VITAL. The Consortium discussed which activities could be reduced with the least impact on the project. We considered that a small reduction in the number of samples taken in the data-gathering WPs would allow funds to be diverted to the fact-finding missions. We calculated that reducing the total number of samples by 15 % would release the necessary 27,500 euros. These details were communicated to our Project Officer Dr. Laurence Moreau, and Nigel Cook and Kris Willems met with her in Brussels on September 24th to discuss them and request approval for them. Dr. Moreau gave her approval in principle, on condition that the changes and particularly the reduction in maximum sample numbers (with the concomitant reduction in consumables budget for the data-gathering laboratories) were approved by the consortium. All details were then approved at the 2nd RDMB meeting (see Annex 1 section D1.5).

Changes in the consortium, if any

No changes were made to the consortium.

List of project meetings, dates and venues

The first Consortium meeting was held on April 7th – 9th 2008 at the Veterinary Research Institute, Brno, Czech Republic.

The 1st Project Advisory Board meeting was held on April 8th 2008 at the Veterinary Research Institute, Brno, Czech Republic.

The dates of the CAT meetings were 7th May 2008, 8th July 2008, 11th September 2008, 11th November 2008, 16th January 2009, and 17th March 2009. The venue was the Central Science Laboratory, York, UK in each instance.

The dates of the RDMB meetings were 17th June 2008 (by webconferencing, 16th September 2008, 21st January 2009. All were held using webconferencing facilities.

Impact of possible deviations from the planned milestones and deliverables

No deviations from the planned milestone “M2.1 All data-gathering laboratories fully prepared with necessary materials and SOPs occurred”; all SOPs were available to each beneficiary through the project website in Month 10.

Any changes to the legal status of any of the beneficiaries, in particular non-profit public bodies, secondary and higher education establishments, research organisations and SMEs

There were no changes in this reporting period.

Development of the Project website

See Work Package 7 report, above (p59).

Use of foreground and dissemination activities during this period

Foreground activities

Standard Operating Procedures (SOPs) for use in the data-gathering activities in WPs 2, 3 and 4 were prepared during the project’s first year. The preliminary draft of the VITAL Code of Good Practice (a Deliverable in the final year of the project) was begun in this year.

The CAT and the RDMB have commenced actively considering where above foreground can be used with respect to collaborative activities, e.g. within the Codex Alimentarius Working Group Viruses’ Code of Hygienic Practice for Control of Viruses in Food (see below), and within the CEN/TC275/WG6/TAG4’s standards for foodborne virus detection.

Dissemination activities

Two presentations on the work of VITAL were given at the Food Micro 2008 (www.foodmicro2008.org) conference on 1st to 4th September 2008 in Aberdeen, UK. The first was given by Dr. Artur Rzeżutka the WP4 Leader, entitled “Monitoring of Food Supply Chains for Human Enteric Viruses”, and the second was given by Professor Kris Willems the WP6 Leader, entitled “Control of Viruses in Food Supply Chains”.

A poster presentation entitled was presented by Dr. Marta Hernandez-Perez, at the COST 929 Symposium “Current Developments in Food and Environmental Virology”, held at the University of Pisa, Italy, on 9th – 11th October 2008.

Communication between beneficiaries

This is an area where, in general, improvement could be made within the project. All WP leaders and other key project members were given a licence for hosting the net-based conferencing facility, but hitherto on only one occasion has it been used outwith the RDMB meetings. The web-conferencing hosts have been exhorted to make full use of this facility, and training has been given.

Possible co-operation with other projects/programmes

The Working Group on Viruses in Foods, which has been established by WHO / FAO is now, under the auspices of Codex Alimentarius, preparing a Code of Hygienic Practice for Control of Viruses in Food. VITAL is actively working towards the production of a similar guidance document, in WP7. At least 3 individual participants in the VITAL Consortium are also participating in the Codex group. This independent participation could raise issues of project confidentiality, and will need circumspection by participants and careful management by the CAT. All participants have been reminded by the VITAL Legal Officer of the relevant sections within the Consortium Agreement. VITAL would like to work in synergy with the Codex group, so that duplication of international overlapping effort is avoided, and more importantly so that our Code of Good Practice has an enhanced and assured impact among food safety practitioners. Currently, DG SANCO has proposed that the VITAL Coordinator accompanies their representatives to the Codex meetings. VITAL trusts that a future fruitful collaboration with Codex will result in enhanced progress towards our common goal.

The efforts towards production of harmonised and validated SOPs for detection of viruses in foods should be of relevance and interest to the activities of the CEN/TC275/WG6/TAG4 group. In the forthcoming months of VITAL, consideration will be given to approaching this group with regard to sharing the information gained from the validation exercises, and the experiences gained from the practical application of detection methodology in actual analysis of the food supply chain.

Annex 1 - VITAL Deliverables delivered in the first year

D1.1

Project Advisory Board Meeting

D1.2

1st Core Administration Team Meeting

D1.3

1st Meeting of the Research and Dissemination Management Board

D1.4

2nd Core Administration Team Meeting

D1.5

2nd Meeting of the Research and Dissemination Management Board

D1.6

3rd Core Administration Team Meeting

D1.7

4th Core Administration Team Meeting

D1.8

3rd Meeting of the Research and Dissemination Management Board

D1.9

5th Core Administration Team Meeting

D5.2

A Tool for Data Analysis

D7.1

VITAL website

D1.1

Project Advisory Board Meeting

APRIL 7TH 2008

Veterinary Research Institute, Brno, Czech Republic

MINUTES

Present Dr Peter Wyn-Jones (UWA, Chair)*, Dr Jan Vinjé* (CDC), Dr Nigel Cook (CSL, VITAL Co-ordinator), Dr Franco Ruggeri (ISS, Vice-Co-ordinator).
(*external member)

1. Welcome and Introductions

1.1. The Chairman welcomed members to the first Meeting of the Board. He said the Project Kick-Off Meeting had started well and there were good signs for a successful Project.

2. Apologies – Prof Clive Thomson, Dr Serve Notermans, Dr Marta Hugas

3. Agenda

3.1. No agenda had been set prior to the Meeting so a short list of items to be discussed was agreed.

4. Terms of Reference

4.1. It was agreed that the PAB would review progress, act as a point of contact for the project team and be available to advise in case of technical or managerial difficulties. The Board is not acting on behalf of the Commission. It would be appropriate that it maintained a distance from the day-to-day activities of the Project so that a disinterested view could be held when required. Minutes of the Board will be provided to the Co-ordinator by the PAB Chairman.

4.2. PAB reports will be written to coincide with major Project Team meetings and published according to the VITAL Description of Work.

5. Issues which require addressing

5.1. Several issues arose from the main meeting and the workshop which, in the view of the Board, are critical to the success of the Project and must be addressed as a matter of some urgency, since they affect the early (as well as later) stages of the work.

5.2. Quality Management System (QMS). Currently there is no documented QMS for the Project. This needs to be put in place and an individual (Quality Manager, (QM)

needs to oversee this part of the project. It would be especially important to have a QMS in place should the Commission undertake a technical fact-finding missions at any time.

- 5.3. Reference materials need to be identified which can be used as (a) training aids and (b) positive controls in the data-gathering phases. It was suggested that one (preferably RNA) virus should be used as process control, as was discussed in the main meeting, but individual (RT-)PCR controls and IACs are also needed for each target virus. These materials need to be obtained and (where appropriate) distributed/propagated in the data-gathering laboratories.
- 5.4. Funding If necessary, funding for implementing a QMS should be drawn from appropriate parts of the budget – this may necessitate a revision of the numbers of samples that can ultimately be processed and reconsideration of the data for the quantitative virological risk assessment (QVRA).
- 5.5. Competence assessment The QMS should include a decision-making process for assessing competence; this is not so much as to score laboratories as to ensure that they are competent to process samples and carry out the detection method with confidence, so that they know that the data they obtain in field conditions is reliable. Proven efficiency of data-gathering laboratories is essential. If it is necessary to extend the preparatory phase to achieve this than this should be done. The model provided by VIROBATHE could be used. The time currently allocated for the data-gathering laboratories to achieve proficiency in the methods (three months, starting 1.4.08) is too short, six months should be allowed and if a laboratory can become proficient in less than that time then it should be allowed to commence sampling; if a laboratory cannot be proficient in 4-5 months then a trouble-shooting visit should be arranged in which either a scientist from the laboratory in question visits an expert laboratory (RIVM or UB were suggested) or *vice versa*.
- 5.6. The focus in the preparatory phase should be on adenoviruses, though experience with HEV will need to be gained later.
- 5.7. Methods protocols These must be finalised as soon as possible; this will involve discussion between the Co-ordinator and the Leader of WP 2 but there will also need to be crosstalk between laboratories to ensure everyone knows what is decided; it may be useful to create and update a chart such as the example given below so that the status of each method/SOP can be seen easily, e.g.

		HAV	HEV	NOV	ADV
Food sampling	farm	√	√	etc	
	producer				
	sale				
Sample processing	farm				
	producer				
	sale				
Virus detection	farm				
	producer				
	sale				

It was understood from the main workshop that CEN TAG 4 draft methods are to be used, but later discussions appeared to indicate that methods as given in original

papers from which TAG4 drafts were derived, will be used. This indecision needs to be addressed urgently so that protocols are finalised and proficiency training started. The decisions on which sample process controls will be used must be taken soon.

A short document stating references for each method would be helpful.

- 5.8. The Logframe Spreadsheet could be more user-friendly with regard to visibility of comment boxes.
- 5.9. Feedback from Expert Stakeholders. Several points were raised through the PAB Chairman:
 - 5.9.1. why test blood + liver + meat? It could be better e.g. to take blood + meat from three pigs rather than blood + liver + meat from two pigs
 - 5.9.2. the meaning of "imported foods" should be clarified to Expert Stakeholders; they may not have realised that the term also encompasses foods sourced in same country but outside local area
- 5.10. The Questionnaire in WP6 to each data-gathering laboratory regarding the food production chains that they intend to analyse, and the location they plan to obtain samples from, should be prepared asap so that the producer profiles can be put in place then the risk assessors can review them.

PWJ April 08

D1.2

1st Core Administration Team Meeting

Wednesday 7th May 2008

Minutes and Actions

Attendees:

Nigel Cook (NC)
Martin D'Agostino (MDA)
Christina Steveni (CS)

1. Apologies for absence

- None

2. Minutes of the last meeting and matters arising

- First meeting of CAT therefore no matters to review

3. Communication with the Commission

- NC reported that the first PAB meeting had been held and the deliverable sent to the Commission. The Grant Agreement had not been received and this was causing concern to some of the beneficiaries. **ACTION:** NC to contact Laurence Moreau to ask for an update.

4. Contractual

- Andy Gilbert is ready to send out the final revision of the Consortium Agreement for review and signature. **ACTION:** AG to send out Consortium Agreement

5. Expenditure Monitoring

- The CAT reviewed the expenditure claims submitted and paid to the PAB and Expert Stakeholders. Several of the Expert Stakeholders had yet to submit claims and Nigel Cook agreed to email them to remind them to send claims in by 31st May **ACTION:** NC to email Expert Stakeholders with reminder to submit expenditure claims.
- It was hoped to be able to invite the Expert Stakeholders to the second project meeting but there were not funds in the budget so funding depended on travel budget savings.
- Bank details had still to be received from partners 3,6 and 7. **ACTION:** CS to issue reminder to send in bank details.

6. Legal

- The TAG4 methods were still being developed and use of these methods was dependent on signing a confidentiality agreement that could hinder publications. It had therefore been decided at the project meeting to use core published methods based on the TAG4 methods and to develop SOP's for use in VITAL.

7. Ethical

- Marta Hernández Pérez is formulating an action plan to ensure that VITAL complies with EU policies.

8. Communication with PAB

- The first PAB meeting was held and the report disseminated to the Commission.

9. Internal Communication

- The VITAL website was under construction. It was decided that NC and MDA would review development of the website after the meeting.
- The Netbased conference system had been purchased and a couple of practice session had been held. There were some technical issues that were being addressed and it was hoped that this would soon be resolved.

10. Actions from PAB

- The budget needed to be agreed and the fact-finding missions scheduled. **ACTION:** Kris Willems
- The SOPs needed to be finalised. **ACTION:** NC to contact authors.
- A quality manager needed to be appointed. **ACTION:** NC to appoint a quality manager.

11. AOB

- None

12. Date of Next Meeting

- The next CAT meeting will be held on **8 July 2008** in room **12G46**.

D1.3

1st Meeting of the Research and Dissemination Management Board

17th June 2008, by webconferencing.

Minutes

Present

Nigel Cook, Martin D'Agostino, Andy Gilbert (Defra CSL)
Kris Willems (KULeuven)
Petra Vasickova (VRI)
Leena Maunula (UH)
Apostolos Vantarakis (UP)
Franco Ruggeri (ISS)
Ana Maria de Roda Husman, Saskia Rutjes, Martijn Bouwknegt (RIVM)
Wim van der Poel (WUR)
Artur Rzezutka (NVRI)
Tamas Petrovic (NIV-NS)
Peter Raspor (UL-BF)
Marta Hernandez (ITACyL)
Rosina Girones, Anna Carratala (UB)

Apologies

Malcolm Banks (Defra VLA)

The Grant Agreement

Copies of the GA were sent to all beneficiaries on 12th June. All beneficiaries have now reported receipt. 3 copies of Annex IV, Form A must be signed, and returned to the Coordinator so that he receives them by Friday 27th June. It was made clear that if any beneficiary defaults on this deadline it will seriously compromise their participation in VITAL. **Action: All VITAL Beneficiaries**

The Consortium Agreement

Copies of the CA were sent out to all beneficiaries on 12th June. All beneficiaries have now reported receipt. The authorised signatory of each institute must sign at the appropriate page in Section 13 (e.g. for UL-BF page 31); 2 signed copies should be sent to the Coordinator so that he receives them by Tuesday 1st July. For Attachment 5, please ignore previous instructions (the Coordinator apologises for any confusion); instead, please send via email the following details of the senior scientist involved in VITAL:

Name
Position
Tel. +
Fax +
E-mail

Each beneficiary should read the Material Transfer Agreement (MTA) which UB requires to release SOPs to VITAL, and then confirm to the Coordinator that they are content that he signs on behalf of the Consortium. Beneficiaries themselves do not have to sign the MTA (apologies for previous request for signatures). **Action: All VITAL Beneficiaries**

Progress of Workpackage 2

WUR have drafted the SOPs, which have revised by UB, RIVM and ITACyL. David Rodriguez-Lazaro will complete the draft SOPs by the end of June, and circulate to beneficiaries for comment. **Action: ITACyL**

ITACyL has nearly completed the construction of the internal amplification controls (IACs). They require virus nucleic acids so that the optimisation of the IAC concentrations can be performed for each assay. **Action: UB and RIVM**

Murine norovirus (muNv) has been chosen as the sample process control. UB and ISS can supply muNv and the cell line required for its propagation, but material transfer agreements (MTAs) need to be signed on behalf of the Consortium by the Coordinator before any materials can be sent out. UB and ISS to send the MTAs to the Coordinator, who will circulate them to the Consortium for confirmation they are content he signs them on their behalf. **Action: UB and ISS, followed by CSL followed by all beneficiaries**

MuNv will be used for quality control in the project. Quality control will be performed by the data-gathering partners as described on Page 17 of the VITAL Description of Work (DoW). We still need to have a Quality Manager (QM) as advised by the PAB. **Action: a volunteer is requested for the QM role**

Rosina Girones has contacted the organization QCMD (www.qcmd.org), a not-for-profit organisation involved in quality control of molecular diagnostics. They stated that they can prepare a program only for VITAL according to the projects needs. This may be a very good, rapid and cheap way to organize intercalibration and proficiency exercises and also to distribute to the labs reference materials. They have materials for adenoviruses and noroviruses, and can send the materials to the labs for only about 325 Euros per lab. The schedule could be: as soon as the labs have the SOPs and by all the reagents needed, labs may start practicing with a plasmid for example (or a viral suspension) and if it works can then start the evaluation of the standard materials send by QCMD. Results are entered on-line and very quickly the labs may get the correct answers to evaluate their performance and also the coordinator may have a report of all labs together. The materials sent by QCMD may be used also as standard materials. We will need further information on what exactly QCMD could provide for VITAL, before any decision is made. **Action: UB**

As discussed in Brno (please see Kick-Off Meeting Summary, sent on April 22nd), there will be ring trials to allow each Data-Gathering Laboratory (DGLs) to demonstrate competence in the method(s). Following today's discussion, the first trials will comprise:

- The SOP for soft fruit, with muNv
- The SOP for pork products, with muNv

We may then hold subsequent trials using the adenovirus and norovirus reference materials discussed above. When the SOPs are finalised and sent to the DGLs, and they have the methods for detection of muNv in place, the ring trials will commence. CSL will oversee the ring trials, and will begin to prepare the SOP for the trial this month. **Action: CSL**

Colleagues at RIVM requested that they are copied into all correspondence concerning SOPs, quality controls, and ring trials. This is so that they can provide timely advice to ensure that the final methodology is compatible with optimal data analyses.

Progress of WP 5

Progress towards Deliverable 5.1 “A guidance document on data collection and analysis” is on track for completion in Month 12.

The software tool for data analysis (D5.2, due Month 8) is now under development, in Excel spreadsheet format. The data to be analysed will focus on elimination and reduction of viruses through processing treatment. A detailed document on how to enter the data will be made available alongside the tool.

Deliverable 5.3 “A Document on available risk assessment models to be able to meet the required criteria for Quantitative Viral Risk Analysis” is linked to Task 5.4. The relevant scientific literature is being examined, along with unpublished information at RIVM. The appropriate criteria for quantitative analysis of viruses in foods will be extracted. A publication is expected to accrue from this Task.

Progress of WP6

A draft questionnaire has been sent out by KULeuven to the DGLs regarding the current practises being followed by farms and processors from which they plan to take samples. Background review questionnaires should be completed for 3 farms / premises by each DGL. The completed background review questionnaires will be used by KULeuven to select 1 company or farm for each DGL, and then a fact-finding mission will be performed. The fact-finding mission will be linked to sampling, so that the sampling points are realistic to the production situation. The first questionnaire involves fruit and vegetable production, and all DGLs have sent comments on the draft. A second draft questionnaire involving pork production has now been sent out. Comments on the pork production draft, should be returned to Kris Willems (kwi@scientiaterrae.org) as soon as possible. **Action Defra VLA, VRI, UH, UPA, ISS, NVRI, NIV-NS and ITACyl**

A guidance document on how to use the questionnaire will be prepared and sent out by the end of June. **Action: KULeuven**

Each DGL should send the name of the person who will be performing the questionnaire, and their relevant experience, to Kris. **Action: Defra VLA, VRI, UH, UPA, ISS, NVRI, NIV-NS and ITACyL**

Not all parts of the background review questionnaires will need to be completed by each DGL, for instance if any question is not applicable a N/A should be entered. Space for comments is available on each questionnaire, and should be used to provide as much relevant information as possible. Kris will contact anyone with problems about the questionnaire, or questions regarding the companies, on a bilateral basis. Kris will also organise a training session, by the webconferencing facility.

After all the information from the background review questionnaires has been collated, KULeuven and UL-BF will perform a series of fact-finding missions to each site. A budget for the visits was prepared by CSL: the total cost of the fact-finding missions will come to 12,540 euros. 12,000 was set aside for BH-UL to perform visits to the Expert Stakeholders, but this will be more productively used to perform these fact-finding missions. KULeuven and UL-BF will prepare a plan for the visits based on this budget. **Action: KULeuven and UL-BF**

Copies of all correspondence regarding WP6 will be sent to RIVM as well to enable timely interaction, which will benefit the risk assessment.

Progress of WP7

The website has now been launched. Martin D'Agostino asked that each beneficiary advise him of any suggestions they may have to develop the site further. **Action: All beneficiaries**

All matters relating to the 2009 Consortium Meeting in Novi Sad are in progress.
Action: NIV-NS

Financial Matters

The first tranche of funding will be released when the Grant Agreement has been signed by the Commission. It is hoped that this will be no later than August 2008. The Coordinator will ask the Project Officer for an update. **Action Defra CSL**

A.O.B.

The Coordinator will contact the Project Officer regarding a possible revision of the project timeplan. This revision will take the form of running WPs 2, 3 and 4 simultaneously, so that they all may take until Month 30 for completion. This will not affect the budget or Logical Framework, and only require putting back 6 deliverables. **Action: Defra CSL**

ITACyL requested that beneficiaries send information on the gender aspects of the project, as requested. All beneficiaries should send the appropriate information to Marta Hernandez (ita-HerPerMa@itacyl.es) as soon as possible. **Action: All beneficiaries**

Dates of future RDMBs

16th September 2008

20th January 2009

1 month before each RDMB, the Coordinator will request a brief Report from each WP Leader. They in turn may wish to request reports from their Task Leaders

D1.4

2nd Core Administration Team Meeting

Tuesday 8th July 2008

Minutes and Actions

Attendees:

Nigel Cook (NC)
Martin D'Agostino (MDA)
Christina Steveni (CS)

1. Apologies for absence

- None

2. Minutes of the last meeting and matters arising

- Minutes and action agreed as a true record. No matters arising.

3. Communication with the Commission

- NC had sent the deliverables that were due to the Commission. The first RDMB meeting had been held and was a success. No other issues with the Commission.

4. Contractual

- The grant agreement had been received on 4th June and sent to the partners on the 12th June asking for them to print off and sign the Accession to the Contract form in triplicate and return to CSL within the 45 day deadline.
- The main grant agreement between CSL and the Commission had been signed and returned within the deadline.
- All partners had signed and returned the Accession to the Contract forms and returned them within the deadline.
- One of the partners - Central Veterinary Institute of Wageningen UR had changed its name to ID-Lelystad. The contract might have to be reissued and NC had been advised to retain the Accession to the Contract forms from partners until contacted by the Commission. It is likely that this will further delay the advance payment.
- The consortium agreement had been sent out with a return date of 1st July. However, the University of Barcelona wanted changes. **ACTION** NC to contact partners to see if they were happy with the changes and would allow their signatures to apply to the next version of the consortium agreement. **ACTION:** Andy Gilbert (AG) to advise

whether the change of name of the partner from Central Veterinary Institute of Wageningen UR to ID-Lelystad needs to be reflected in the consortium agreement.

- The University of Barcelona had requested a Material Transfer agreement between the VITAL consortium and itself. There was debate whether NC should sign on behalf of the consortium or each partner should sign the agreement. **ACTION:** AG to read and advise whether NC should sign on behalf of the consortium or whether each partner needs to sign the agreement. Also AG to advise whether change of name from Central Veterinary Institute of Wageningen UR to ID-Lelystad needs to be reflected in the agreement.
- A material transfer agreement needed to be set up between the consortium and Washington University in the USA for transfer of the murine norovirus. **ACTION:** AG to read and advise whether NC should sign on behalf of the consortium or whether each partner needs to sign the agreement. Also AG to advise whether change of name from Central Veterinary Institute of Wageningen UR to ID-Lelystad needs to be reflected in the agreement.

5. Expenditure Monitoring

- It was agreed that CS would prepare a statement to show how much had been spent on travel for PAB and Stakeholders **ACTION:** CS to issue statement of expenditure on travel for PAB and Stakeholders
- It was agreed that the shipping costs of the Glass Wool sent out by CSL to the partners would be deducted from the advance payment.
- CS to review the bank details and email partners who had yet to send them.

6. Legal

- None

7. Ethical

- Marta Hernández Pérez is formulating an action plan which will be available via the web site.

8. Communication with PAB

- The PAB had made helpful comments on the SOPs
- No recent correspondence from Marta Hugas of EFSA who had indicated she would be willing to be a member of the PAB. **ACTION:** NC to contact Marta Hugas or one of her colleagues.

9. Internal Communication

- The VITAL website was up and running with public and private areas.
- The Netbased conference system had been purchased and two meetings had been held. There were still some technical issues that were being addressed.

10. Actions from PAB

- None

11. AOB

- None

12. Date of Next Meeting

- The next CAT meeting will be held on **11 September 2008** in room **12G46**.

D1.5

2nd Meeting of the Research and Dissemination Management Board

Tuesday 16th September 2008, by webconferencing.

Minutes

Present

Nigel Cook, Martin D'Agostino (Defra CSL)
Kris Willems (KULeuven)
Ivo Pavlik, Petr Kralik (VRI)
Leena Maunula (UH)
Apostolos Vantarakis (UP)
Franco Ruggeri (ISS)
Ana Maria de Roda Husman, Saskia Rutjes (RIVM)
Wim van der Poel (WUR)
Artur Rzezutka (NVRI)
Tamas Petrovic (NIV-NS)
Peter Raspor (UL-BF)
Marta Hernandez (ITACyL)
Rosina Girones, (UB)

Apologies

Malcolm Banks (Defra VLA)

The Grant Agreement

The Grant Agreement has been signed by the European Commission. CSL has sent out copies of the signed GA to all beneficiaries, in September. Beneficiaries are requested to inform Martin D'Agostino when they receive the GA copy. If they have not received the copy by Monday 6th October, they should inform Martin of this.

The Consortium Agreement

The Consortium Agreement was completed and signed by all beneficiaries. Copies of the signed CA were sent to all beneficiaries by CSL in August. Martin D. will contact all beneficiaries to confirm that they received the CA copy.

Proposed revisions to the work program

Following extensive discussions within the Consortium, and ad hoc meetings between the Coordinator and various participants, it was decided that a revision of the VITAL workplan should be proposed, to take into account the requirement for extensive fact-finding missions to the sampling sites, and the requirement for more extensive development and testing of the

analytical methods than was originally envisaged. Details of the proposed workplan revision are given in Annex 1 to these minutes. These details were communicated to our Project Officer Dr. Laurence Moreau, and Nigel Cook and Kris Willems met with her in Brussels on September 24th to discuss them and request approval for them. Dr. Moreau gave her approval in principle, on condition that the changes and particularly the reduction in maximum sample numbers (with the concomitant reduction in consumables budget for the data-gathering laboratories) were approved by the consortium. Those present at today's RDMB have approved the changes.

A reduction of 15% in sample size will not necessarily affect the quality of data (i.e. estimates for the mean of parameters) in the VITAL setting when the sampling plan remains unaltered, which is the case. What is affected by a reduced number of samples is the uncertainty surrounding the estimated means. A smaller sample size leads to less information about the parameters and thus more uncertainty. Actual effects of the reduction cannot be estimated beforehand, because this is subject to many unpredictable and uncontrollable circumstances. However, simulation studies may give some insight in the magnitude of the effect of reducing the sample size given certain assumptions (Annex 2). The expected increase in uncertainty is present, but may be marginal as shown in Annex 2, although the use of other assumptions may lead to scenarios that give other results. But the (possible marginal) unfavourable effects of sample size reduction are to be compared to the beneficial effects of the production site surveys. By identifying the critical control points, data collection will be focussed more on the problem areas compared to sampling predetermined points, resulting in more realistic modelling of the food chain. The benefit of this aspect outweighs the increased uncertainty in parameter estimates.

Progress of Workpackage 2

During the meeting in Brno it became clear that the CENTAG4 draft methods could not be accepted to be used as the VITAL SOPs, and it was agreed to draft dedicated VITAL SOPs for virus detection in the food production chains. For this purpose extensive work has been undertaken (especially by ITACyL) on drafting of SOPS for virus sampling and concentration, and development of IACS.

An overview of the present status of the work on VITAL SOPs is given in the tables underneath:

A) Status of VITAL virus concentration and nucleic acid extraction SOPs

SOP nr	Matrix	Available/ completed
001	Sampling + virus concentration conc feaces	✓
002	Sampling + virus concentration Hand wash	✓
003	Sampling + virus concentration Animal derived fertilizer	✓
004	Sampling + virus concentration Irrigation waters	✓
005	Sampling + virus concentration Soft fruit	✓
006	Sampling + virus concentration Vegetables	✓
007	Sampling + virus concentration Shellfish	✓
008	Sampling + virus concentration Slaughterhouse effluents	✓
009	Sampling + virus concentration Blood	✓
010	Sampling + virus concentration Liver tissue	✓
011	Sampling + virus concentration Pork meat	✓
012	Nucleic acids extraction (NA) extr. Animal feaces, animal derived fertilizer and animal blood	✓
013	NA extr. Pork liver tissue and meat	✓
014	NA extr soft fruit, shellfish, vegetables	✓
015	Nucleic acids extraction from irrigation water, slaughter house effluents, or harvesters' hands wash-off	✓

B) Status VITAL realtime (RT) PCR protocols

(✓= available / completed)

Virus	Primers and probes	Internal amplification Control (IAC)
Norovirus GGI	✓	✓
Norovirus GGII	✓	✓
HAV	✓	✓
HEV	✓	✓
Porcine Adenovirus	To be delivered by partner UB	Waiting for target sequence
Human Adenovirus	To be delivered by partner UB	Waiting for target sequence
Bovine Polyomavirus	To be delivered by partner UB	Waiting for target sequence
Mouse Endomyocarditis virus	? Choice of virus to be confirmed;	? availability of IAC to be investigated

C) NB realtime (RT)PCR kits need to be selected

To enable the use of SOPs for index viruses (Adenoviruses and Bovine Polyomavirus) MTAs with the University of Barcelona still have to be signed by some beneficiaries.

The SOP for QPCR of human adenovirus is that which was used in the VIROBATHE project. The VIROBATHE leader Dr. Peter Wyn-Jones has previously kindly agreed to release this SOP to the VITAL consortium under confidentiality, so it is available now for use. An issue

appears to have arisen regarding the selectivity of this assay; Rosina has reported that it is not fully selective for human adenovirus strains, and that some animal adenovirus strains may also be detected. Further information is required here, and those members of the Consortium who will attend the ENVIRONET meeting in Pisa 9th- 11th October are requested to have a brief informal meeting there to discuss the information.

The suggestion has been made to use murine endomyocarditis virus as an alternative to murine norovirus as a sample process control. If there are any objections to the use of this virus, please inform Wim by Monday October 13th.

Nucleic acid extraction will be performed using a commercial kit, e.g. QIAGEN DNA minikit. All data-gathering laboratories are please to inform Wim of their preferred choice of kit, by Monday October 6th.

Material Transfer Agreements

The University of Barcelona has requested that MTAs are signed before the SOPs for the QPCR assays for porcine adenovirus and bovine polyomavirus are released to the Consortium. All beneficiaries except KULeuven and RIVM have now signed the MTA's. The U of Barcelona has indicated that they are willing to enter into individual Agreements with the 2 dissenting beneficiaries. KULeuven and RIVM are therefore requested to approach UB individually, and negotiate the MTA revisions individually. Meanwhile, UB has indicated that it would not insist on a complete Consortium MTA, and when the Agreements signed by the other beneficiaries are sent to them, they will be happy to release the SOPs to those beneficiaries. CSL will compile the signed MTAs and send them to UB in due course. *The Coordinator is concerned that these MTAs may in any case not have been necessary, as we are all bound under the confidentiality clauses in the VITAL Consortium Agreement.*

Washington University has requested that the Consortium signs an MTA before they will allow us to use murine norovirus. Again, all beneficiaries have signed the MTA save KULeuven and RIVM. In this case however Washington University have refused to allow individual agreements. This has delayed the acquisition of the sample process control virus, and we must identify a new candidate (see WP2 above) to allow the work to proceed with minimal further delay. However, the use of murine norovirus would be highly beneficial to the survival studies in Task 6.2, and the dissenting beneficiaries are strongly requested to resolve this problem with their legal teams. Please see Andy Gilbert's comments, in Annex 3.

Quality control

Following the recommendations of the Project Advisory Board regarding quality control of the analytical work in WPs 2-4, the Coordinator has been in discussion with Dr. Paul Wallace of the company Quality Control for Molecular Diagnostics (www.qcmd.org). He is drafting a plan, with costings, for supplying VITAL with QC materials and analysing results of a quality control program. The Commission's Project and Financial Officers have stated that we can use this company as a supplier, and that there would be no subcontractual issues attached.

The ring trials will be conducted solely by VITAL. The coordination of the trials, and the statistical analyses of the results, can be performed by CSL. The trial outlines will be prepared by end-November, and the trials themselves should be provisionally scheduled to commence end-January 2009.

Progress of WP3 and WP4

Data-gathering laboratories have completed the background review questionnaires for decision of the critical points. They are in the process of completion of the identification of processing plants (3 sites per data mining country) and retail premises. For each WP, data-gathering labs need to approach the managers of premises regarding the fact-finding missions, to ensure that they are provided with the information required for the realisation of the WPs, and that they will be open to receive the fact-finding mission team.

Progress of WP 5

Task 5.3 MPRM development

VITAL involves different food chains and the transmission routes of viruses to humans differ per chain. Therefore, each of the models describing these routes will require a different chain of modules in the risk models. In the first 6 months of VITAL, the modules for norovirus infection due to oyster consumption have been developed. These modules jointly describe the norovirus (NoV) contamination of oysters from moment of harvest through moment of consumption using data on NoV-contamination collected at the RIVM and from literature. The estimated consumed NoV-dose is subsequently related to the dose response model described in literature to characterize the human risk of infection. The currently used data on NoV-contamination of oysters will be replaced by VITAL-data once available to estimate the human NoV infection risk for oyster consumption in Europe.

Furthermore, the modules of this model can be used as baseline for some of the modules for the other food chains.

Task 5.4 Prioritization of risk assessment criteria

Quantitative viral risk assessment and quantitative bacterial risk assessment differ in approach with respect to quantification of pathogen concentration, data needs and dose response models due to the different characteristics of the pathogens. Several of the differences in approach have been recognized during the work performed under Task 5.3. Furthermore, prior conducted risk assessments yielded experience on this matter. The findings will be drafted in a report (rolling revision) that will be supplemented throughout the development of the ViTAL QVRA.

Data-gathering laboratories are requested to provide to RIVM consumption data from their specific regions regarding soft fruit, salad vegetables, pork products and shellfish. Ana Maria will send out an email stating exactly what is required, in due course.

Progress of WP6

Task 6.1 Critical evaluation of current HACCP systems

The background review questionnaires for the soft fruit and salad vegetable food chains have been completed. A consensus is needed for the format of the questionnaire for the pork supply chain, and ISS and VLA are requested to contact Kris as soon as possible to discuss the finalisation of the format. Background review questionnaires for processing plants and retail premises are still to be formulated.

The data-gathering laboratories are requested to finalise the sampling schedules by the end of January 2009. This will allow the fact-finding mission team to plan the necessary visits.

T6.2 Survival and elimination of viruses.

A student is in place at the University of Barcelona to study adenovirus. A student is expected to start in RIVM on 1st November to study norovirus. VLA/WUR are still interviewing potential candidates.

Progress of WP7

The first two Tasks in this WP are scheduled to commence later in the project. However, planning of the Symposium Workshop and Training Course can commence earlier with the decision on the venue. Apostolos and Peter will decide this, and communicate the decision for the next RDMB in January.

Update on 2nd Annual Consortium Meeting

The next Annual Consortium Meeting will be held at the Scientific Research Institute at Novi Sad in Serbia, on 6th – 8th April 2008. The first day will be an overview of project progress. On the 2nd day the Data-Gathering Workshop for all partners will be held (Task 5.1). On the 3rd day there will be a tour including visits to local production and sampling sites. Colleagues are advised to make their travel plans as early as possible to keep costs low.

Financial Matters

The devolved budget for the fact-finding missions can be split between KULeuven and UL-BF. UL-BF is already allocated 12,000 € for Task 6I. and KULeuven 500 €. Therefore an extra 8,000 € will be allocated to UL-BF and 19,500 to KULeuven. Christina will advise on how this allocation is to be administered.

A.O.B.

None

Date of next RDMB

The next meeting of the Board will be held by webconference on 20th January 2009.

The proposed revision to the workplan of the VITAL project

The proposed revision focuses on the development of the analytical methods, the gathering of data through taking and analysing food and environmental samples, and the development of the HACCP recommendations and Code of Good Practice. The proposed revision affects WPs 2-4, and some parts of Tasks T6.1 (HACCP evaluation) and T6.4 (HACCP models). WP1, WP 5, Task 6.2 (survival studies), Task 6.4 (vaccination study), and WP7 will remain unchanged.

Briefly, the proposed revision is:

- 1. The method development and validation phase (Task 2.1) will now extend until March 2009.**
- 2. The methods will be validated through ring trial early 2009.**
- 3. Fact-finding missions to each sampling site will be performed after the methods have been validated. The visits will commence in April 2009.**
- 4. Samples be taken during or immediately after the fact-finding missions, and analysed subsequently.**
- 5. The cost of the fact-finding missions (40,000 €) will require a 15 % reduction in the number of samples processed.**

The reasons for proposing this revision, and further details of the proposed revision, are detailed below.

Development of methods

During the preparation of the proposal, it was envisaged that the SOPs for extraction and detection of viruses from food and related samples would be available in early 2008. However, our information from TAG 4 in April was that these SOPs would not be published until 2012. Therefore it has been necessary to develop our own SOPs for most methods.

For 2 of the index viruses, however, there is an issue regarding the use of real-time PCR assays for their detection. Methods exist which have been developed by the University of Barcelona (a VITAL beneficiary) previous to the project. However, the University wishes to patent these methods, and will not release the SOPs to us until we have signed a Material Transfer Agreement. Most VITAL beneficiaries have signed, but 2 institutes have asked for modifications to the Agreement. This has delayed the acquisition of these necessary methods. The VITAL Coordinator has recently reached an understanding with the University of Barcelona to allow the Agreement to be signed on an individual basis by beneficiaries.

Another issue has arisen over the use of the sample process control virus. The TAG4 group is proposing to use a genetically modified virus, but this will not be acceptable by all VITAL laboratories. We chose as our candidate control virus murine norovirus. It has transpired that this virus can only be obtained from a US University, Washington University, which asked that a Material Transfer Agreement be signed. Again, 2 institutes required amendments to this Agreement, and Washington University have refused to allow individual Agreements, insisting on an Agreement signed by the whole consortium. To overcome this delay, we are currently choosing a new control virus which has no associated legislative or legal issues attached.

Otherwise, most of the SOPs which have been developed are now ready for testing. Because of the extra work we have had to do to revise the methods and identify new control viruses, we request that Task 2.1 is extended until March 31st 2009. This will:

- Allow the SOPs to be trialled by each data-gathering laboratory, to become familiar with them, until end-2008.
- Conduct ring trials of selected methods will in January - March 2009. This will validate the methods, demonstrate that they are fit for purpose, and demonstrate then capability of each data-gathering lab to perform the methods. The methods will then be fully acceptable for use in the data-gathering phases.

It is essential for the outcome of VITAL to perform full fact-finding missions for the production and processing phases, at the farms and factories from which the food samples will be taken in WPs 2-4. This is essential:

1. So that the sampling points chosen will reflect any risk of virus contamination, and embody the points at which contamination may be controlled.
 2. To fully inform the Code of Good Practice, as it will be drawn up following an accepted standard auditing procedure.
- Fact-finding missions will be performed at each site identified by data-gathering laboratory (farm, processing premise, retail premise).
 - Sampling of sites will take place only after fact-finding missions are performed.
 - The data-gathering laboratory will determine the time when the site should be sampled, then contact the VITAL Fact-finding mission Team giving them at least 1 week's notice. The fact-finding mission Team will visit the site, identify the points to be sampled, and samples will be taken by members of the data-gathering laboratory.

All data-gathering methods and SOPs should be tested and validated through ring trail before the fact-finding missions are performed.

- Data-gathering will begin full-scale immediately after the next Consortium Meeting in April 2009. Some site samples will however be taken for the ring trail, and the results of their analysis will be incorporated into the overall VITAL data analysis.

In consequence, this would truly fulfil the aim of the project to integrate monitoring and control of foodborne viruses within food supply chains.

Because the fact-finding missions will now be fully integrated with the sampling plans, the visits will be more extensive and frequent. We would like to allocate 40,000 euros for the fact-finding missions. We have 12,500 euros for Task T6.1 already, but we need to find the extra 27,500 euros from other areas within VITAL. The Consortium has discussed which activities could be reduced with the least impact on the project. We considered that a small reduction in the number of samples taken in the data-gathering WPs would allow funds to be diverted to the fact-finding missions visits. We have calculated that reducing the total number of samples by 15 % would release the necessary 27,500 euros, and we request that aspect in the revision.

Budget calculations regarding reducing the number of samples taken in VITAL to release €27,500

Each analysis of salad vegetables, shellfish and soft fruit supply chain samples has been budgeted at € 65. There are 2160 of these, totaling € 140,400.

Each analysis of pork supply chain samples has been budgeted at € 45. There are 1005 of these, totaling € 45,225.

Total sample budget = € 185,625.

€27,500 is 14.8 % of € 185,625, therefore if the total number of samples in each grouping were reduced by 15 %, the sum required to support the fact-finding missions would be released.

Effects of sample size reduction

In general, samples are taken to obtain a mean parameter value and its variability. The more samples analyzed, the more information is obtained. More information yields a higher precision and certainty for the parameter estimates (given a proper study design). With the expected number of samples collected in VITAL, the estimated mean for parameters is not expected to be affected by the sample size reduction, because of random sampling. Reducing the number of samples will mainly increase the uncertainty for parameter estimates. The magnitude of this increase cannot be determined beforehand, but simulation studies may give some insight when desired. The two figures below illustrate the effect of a sample size reduction of 15% for hypothetical estimates of virus concentration in irrigation water (Figure 1) and for HEV prevalence on pig farms (Figure 2). In these simulations, the VITAL DoW was followed, in which it is proposed originally to collect 30 samples per beneficiary per critical control point.

Figure 1 shows that the uncertainty in estimated virus concentration per litre irrigation water is larger for the reduced sample size-scenario, leading to a wider distribution of possible values. This scenario holds true when particles are assumed to be distributed homogeneously in the water. The magnitude of the currently simulated effect a reduced sample size, however, is marginal, leading to slightly larger 95% intervals.

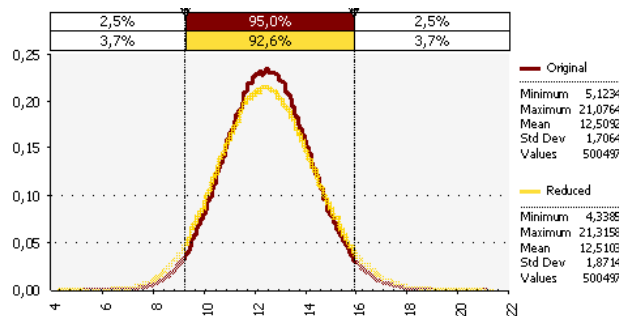


Fig. 1 The variation in estimated concentration for the original sample size ($n=30$) and the reduced sample size ($n=25$).

The second scenario involves an estimate for the apparent prevalence of a virus in a population (*e.g.* for HEV on pig farms). A sample size reduction of 15% will increase the approximated error for the estimate with 1.7% (Figure 2). For instance, when a prevalence of 50% is estimated from 30 faecal samples taken randomly at a pig farm the expected confidence interval ranges from about 32% to 68%. For 25 samples, this will be from about 30% to 70%. Thus, also in this case the simulated effect of the reduced sample size is marginal.

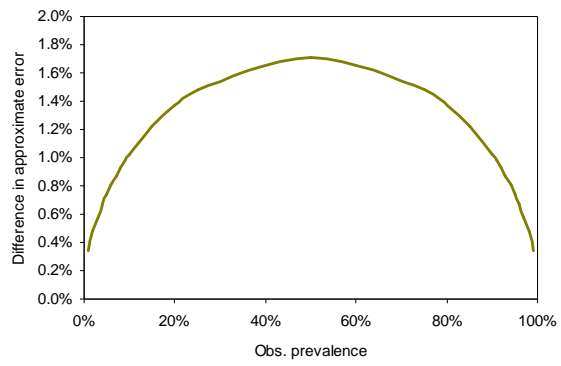


Fig. 2 The error for the estimated prevalence for the original sample size ($n=30$) and the reduced sample size ($n=25$).

[ANNEX 3]

Advice of VITAL's legal advisor Dr. Andy Gilbert, to Coordinator regarding the issue over Washington University's Material Transfer Agreement for murine norovirus

The virus and cell line needed to be stocked and used by VITAL requires an MTA to be signed, by all VITAL members, to satisfy Washington University. The WU position is reasonable in that they need US law to apply to the MTA and will not accept individual variations to the MTA (e.g. Dutch law for RIVM). Please note that you cannot sign on behalf of the consortium (in the absence of a separate power of attorney being granted to you by VITAL members to this effect) in view of clause 7 of the Consortium agreement. WU will need individual signatures on the common form of MTA. I note that you have a majority already signed up.

I cannot suggest any way around this dilemma - save that those VITAL members unwilling to sign are excluded from the research involving the WU virus/cell line. That may be impractical from the research objectives viewpoint, and such an arrangement may well be unacceptable to WU, who may have reasonable concerns that data sharing provisions within the consortium could compromise the undertakings given within the MTA.

My best proposal is that you try to persuade those VITAL members concerned to relax their objections. In particular, reluctance to accept US law in this singular instance seems unreasonable to me. Submission to a foreign jurisdiction would in no way compromise a nation state's general autonomy as to governing law - but would only serve to safeguard the US suppliers interests. It is notable that VITAL is already governed by Belgian law, as any EC Contract would be. So the principle of foreign jurisdiction is already established.

Failing to get common agreement within VITAL on this matter could, I assume, jeopardise the aims of the research effort. For that reason, you may need to consider the voting rights available to the VITAL Research and Dissemination Management Board who, albeit reluctantly, may need to consider what steps may be needed in order to safeguard the reasonable aspirations of those VITAL members willing to sign up to the WU MTA.

D1.6

3rd Core Administration Team Meeting

Thursday 11th September 2008

Minutes and Actions

Attendees:

Nigel Cook (NC)
Christina Steveni (CS)

1. Apologies for absence

- Martin D'Agostino (MDA)

2. Minutes of the last meeting and matters arising

- Minutes and action agreed as a true record. No matters arising.

3. Communication with the Commission

- NC had arranged to meet Laurence Moreau on 24th September to discuss the necessary revision to the work plan and to seek agreement for the fact-finding missions plan.

4. Contractual

- All partners had signed and returned the Accession to the Contract forms and returned them within the deadline. These had been signed by CSL and would be returned to the partners. **ACTION:** CS to return the signed Accession to the Contract forms to the partners
- The University of Barcelona had requested a Material Transfer agreement between the VITAL consortium and itself. There was debate whether NC should sign on behalf of the consortium or each partner should sign the agreement. **ACTION:** AG to read and advise whether NC should sign on behalf of the consortium or whether each partner needs to sign the agreement. Also AG to advise whether change of name from Central Veterinary Institute of Wageningen UR to ID-Lelystad needs to be reflected in the agreement.
- A material transfer agreement needed to be set up between the consortium and Washington University in the USA for transfer of the murine norovirus. **ACTION:** AG to read and advise whether NC should sign on behalf of the consortium or whether each partner needs to sign the agreement. Also AG to advise whether change of name from Central Veterinary Institute of Wageningen UR to ID-Lelystad needs to be reflected in the agreement.
- Both of these issues with the material transfer agreements would be discussed at the next Research and Dissemination Management Board (RDMB).

- The advance had been received and was being paid to the partners. **ACTION:** CS to calculate and pay advances to partners

5. Expenditure Monitoring

- It was agreed that CS would prepare a statement to show how much had been spent on travel for PAB and Stakeholders **ACTION:** CS to issue statement of expenditure on travel for PAB and Stakeholders

6. Legal

- None

7. Ethical

- Marta Hernández Pérez is formulating an action plan which will be available via the web site.

8. Communication with PAB

- No recent communications had been held with the PAB

9. Internal Communication

- The Netbased conference system had been purchased and two meetings had been held. It was now working well.

10. Actions from PAB

- None

11. AOB

- None

12. Date of Next Meeting

- The next CAT meeting will be held on **11 November 2008** in room **12G46**.

D1.7

4th Core Administration Team Meeting

Tuesday 11th November 2008

Minutes and Actions

Attendees:

Nigel Cook (NC)
Christina Steveni (CS)
Martin D'Agostino (MDA)

1. Apologies for absence

- None

2. Minutes of the last meeting and matters arising

- Minutes and action agreed as a true record. No matters arising.

3. Communication with the Commission

- NC had meet Laurence Moreau on 24th September with Kris Willems to discuss the necessary revision to the work plan and to got agreement for budget changes to fund the fact-finding missions plan. The minutes of the second RDMB meeting had the full account of this meeting.

4. Contractual

- All partners had an original Accession to the Contract forms and a full copy of the grant agreement. They were also available on the website.
- The University of Barcelona had requested a Material Transfer agreement between the VITAL consortium and itself. This was signed by all members of the consortium except KULeuven. **ACTION:** KULeuven to sign the material transfer agreement with the University of Barcelona. **ACTION** Barcelona to release SOP
- A material transfer agreement needed to be set up between the consortium and Washington University in the USA for transfer of the murine norovirus. **ACTION:** Partners to send revisions for incorporation into MTA. Responses were still outstanding from two partners. **ACTION:** NC and MDA to talk to AG to discuss how to move obtaining the MTA forwards
- Reporting was discussed. Due to delays in obtaining MTA and the budget reallocations for the fact-finding missions it was becoming important to check that all work packages were still on schedule to have completed all their tasks, deliverables and milestones due at the first reporting period at the end of March 2009. **ACTION:** Partners to report to Workpackage Leaders on their current delivery split down by

tasks. WPLs to produce a quarterly WP report split down by tasks and including information on milestones and deliverables due and progress made. This report is due before the next RDMB meeting on 20th January 2008. **ACTION** WPL will be required to bring a full Annual Scientific Report of their WP, split down by tasks, to the meeting in Serbia, on a USB stick.

5. Expenditure Monitoring

- There will be funds available to offer each Expert Stakeholder 500 € towards the cost of travel and subsistence for the 2nd Annual Meeting in April in Novi Sad.

6. Legal

- None

7. Ethical

- Marta Hernández Pérez is formulating an action plan which will be available via the web site.

8. Communication with PAB

- No recent communications had been held with the PAB

9. Internal Communication

- Nothing to report

10. Actions from PAB

- Appoint quality manager. It had been decided that each WP leader would be a quality manager. **ACTION:** Nigel Cook and Martin D'Agostino to produce a quality sheet, and place on website for comment. Leaders of the data-gathering WPs will be required to do the QC monitoring, during the analytical phases of their WPs.

11. AOB

- None

12. Date of Next Meeting

- The next CAT meeting will be held on **15 January 2009** in room **12G46**.

D1.8

Minutes of the 3rd Meeting of the Research and Dissemination Management Board

10:00 GMT 21st January 2009, by webconferencing

Present

CSL N. Cook, M.D'Agostino
VLA – M. Banks, F. Martelli
KULeuven – K. Willems
VRI – P.Kralik, P. Vasickova
UH – L. Maunula
UPA – A. Vantarakis
ISS – F. Ruggeri
RIVM – S. Rutjes, M. Bouwknecht, K. Verhaelen
WUR – W. van der Poel
NVRI – A. Rzezutka
NIV-NS – T. Petrovic
UL-BF – K. Kovacs, M. Ambrozic
ITACyL – D. Rodriguez-Lazaro
UB – R. Girones

News from the CAT

All contracts, and agreements have been signed and copies placed on website.

Workpackage Progress

Workpackage 2

SOPs

SOPs are available through the website, so partners can practice to work according to these protocols. By the end of Jan 2009 all SOPs must be available so all methods can be tested by the partners.

An overview of the present status of the work on VITAL SOPs is given in the tables underneath:

A) Status VITAL virus concentration and nucleic acid extraction SOPs

SOP nr	Matrix	Available/ completed
001	Sampling + virus concentration conc feaces	✓
002	Sampling + virus concentration Hand wash	✓
003	Sampling + virus concentration Animal derived fertilizer	✓
004	Sampling + virus concentration waters (irrigation water and slaughterhouse effluents)	✓
005	Sampling + virus concentration Soft fruit	✓
006	Sampling + virus concentration Vegetables	✓
007	Sampling + virus concentration Shellfish	✓
008	Sampling + virus concentration Blood	✓
009	Sampling + virus concentration Liver tissue and pork meat	✓
010	Nucleic acids extraction (NA) extr. Animal feaces, animal derived fertilizer and animal blood	✓
011	NA extr. Pork liver tissue and meat	✓
012	NA extr soft fruit, shellfish, vegetables	✓
013	Nucleic acids extraction from irrigation water, slaughter house effluents, or harvesters' hands wash-off	✓

B) Status VITAL realtime (RT) PCR protocols

(✓= available / completed)

SOP nr	Virus	Primers and probes	Internal amplification Control (IAC)
014	Human adenovirus	✓	✓
015	Porcine adenovirus	✓	✓
016	Bovine Polyomavirus	To be delivered by partner UB	Waiting for the oligos sequences
017	Human Adenovirus Nested PCR	✓	✓
018	Norovirus GGI and GGII	✓	✓
019	HAV	✓	✓
020	HEV	✓	✓
021	Murine norovirus	✓	✓

C) (RT)PCR kit of choice : RNA Ultrasens

Any mistakes found by participants in the SOPs are to be notified to Wim or David.

IAC Construction

IACs will be produced in bulk, certified for concentration and purity and shipped to participants through a biotechnology supplier - Yorkshire Bioscience (york-bio.com).

Quality control

The Quality Managers will be the leaders of WP2, 3 and 4. CSL will devise a draft quality worksheet, and circulate it by end-February.

Ring Trials

The ring trials will commence around the end of February. It is considered that the assays themselves should be robust, and therefore the trials will focus on the sample treatment methods. Two archetype methods will be trialled - extraction from soft fruit (SOP 005) and extraction from pork products (SOP 009). The trials will involve testing SOP005 using raspberries spiked with human adenovirus and SOP009 with fresh pork meat (e.g. sausage) also spiked with human adenovirus. ISS will prepare the virus spiking suspensions, and ship to each partner (the shipping costs must be paid by the individual receiving laboratory). Full details of the ring trials will be sent out by the end of January.

ISS will send out samples of murine norovirus to each participant shortly. The cost of shipment must be paid by each individual receiving laboratory.

All the data-gathering laboratories, and those who are hosting students, will participate in the ring trial. Thus the participant labs will be at VLA, VRI, UH, UPA, ISS, RIVM, NVRI, NIV-NS, ITACyL, and UB.

Workpackages 3 and 4

Data gathering laboratories have identified sampling locations (processors and points of sale) of particular food items and have approached the relevant contacts to get permission for the fact-finding missions and the sampling. Each data-gathering laboratory is acquiring the consumption data requested for use within WP4. Each data-gathering laboratory has liaised with Kris to devise the time schedules for the fact-finding missions and sampling.

Workpackage 5

Task 5.3 MPRM development

A request for consumption data has been sent out to all beneficiaries. These data are essential for assessing the human exposure to viruses in the quantitative virological risk assessment. So far, information has been provided from the UK, Poland, the Czech Republic, Serbia, Slovenia and Spain. No response has been received from the other beneficiaries yet.

An Excel tool for data analysis has been created (Deliverable D5.2), based on the concepts of data analysis that have been developed at the RIVM in recent years. A technical guidance document explaining the concept and assumptions will be written before the meeting in Serbia in April of this year. Participants are encouraged to use the tool now on available data, and report to RIVM any problems or questions that arise. The experiences and explanations can be discussed at the Novi Sad meeting. Furthermore, ideas about topics regarding data analysis for viruses should be communicated to RIVM. These topics will then be included in the workshop during the Novi Sad meeting.

Task 5.4 Prioritization of risk assessment criteria

As mentioned in the previous progress report, the prioritization of risk assessment criteria is a continuous process, and the findings are drafted in a report (rolling revision) that will be supplemented throughout the development of the ViTAL QVRA.

Workpackage 6

Finalisation of the background review questionnaires will be done soon.

Provisional sampling schedules have been prepared.

Kris will visit the RIVM shortly to discuss CCPs of virus and sampling schemes in relation to data analysis and risk assessment.

Task 6.2

The PhD-student Katharina Verhaelen has started at the RIVM on January 1st and is currently getting familiar with the required laboratory procedures and NoV.

Workpackage 7

The Women in Science section is now complete and is live on the project website. All MTAs, the Consortium Agreement, the DoW, and the Accession Forms, are now on the Beneficiaries homepage. The SOP page is being updated on a regular basis, and participants are encouraged to check that they have the latest versions. Please also check the Beneficiaries section for the latest minutes of all the meetings.

The provisional plans for the 2nd Consortium Meeting in Novi Sad have been prepared by Tamas and colleagues. A questionnaire on participant attendance has been sent out, for response by 16th February.

Financial Matters

As indicated above, the shipment costs for reference materials sent out by ISS must be picked up by the recipient beneficiaries. Franco will send out an estimate of these costs to each recipient.

Replacement of 2 PAB Members

Dr. Marta Hugas has never responded to any correspondence, and Professor Serve Notermans has informed the Coordinator that he is retiring. The 3 remaining PAB members have been very enthusiastic and active, and could be deemed to be sufficient for the project's needs. A vote was taken on whether to replace D. Hugas and Prof. Notermans, or whether we should reduce the PAB to 3 members. The latter option was carried. The Coordinator will ask Dr. Moreau the Commission's Science Officer to ratify this decision. If she approves, the decision on how to use the money saved will be made at the next RDMB.

Following the RDMB, Peter Wyn-Jones the Chair of the RDMB advised that it may be more prudent to recruit food safety experts from the Expert Stakeholders to the PAB. This would strengthen the Board's ability to give advice on all aspects of the project, and ensure that its focus on practical aspects of food chain safety was maintained. This option will therefore be discussed with Dr. Moreau, and her decision communicated back to the Consortium.

Plan for 1st Annual Report delivery

The absolute deadline for the Coordinator to send the 1st Annual Report is 15th May. Full WP Reports must be delivered by the 6th April. WP Leaders must bring the report on a USB stick or similar to the Consortium Meeting at Novi Sad. These Reports will be reviewed by the Consortium at the meeting.

A.O.B.

Welcome to all the new colleagues, especially the PhD students.

Despite timely requests for each WP Leader to send a brief report for inclusion in the RDMB meeting and minutes, only 3 reports were received. This was not beneficial for anyone, and it has to be remembered that the RDMB minutes are formal project Deliverables which will be scrutinised by the Project Officer. In future therefore it must be insisted that all WP Leaders provide their reports on time, so that they can be collated by CSL into a form that can be discussed during the RDMB. A formal template is now being devised for each WP report, and will be sent out 1 month prior to each RDMB meeting.

Dates of future RDMBs

The next RDMB will take place on Monday 6th April at Novi Sad (time to be decided).

D1.9

5th Core Administration Team Meeting

Friday 16th January 2009

Minutes and Actions

Attendees:

Nigel Cook (NC)
Christina Steveni (CS)
Martin D'Agostino (MDA)

1. Apologies for absence

- None

2. Minutes of the last meeting and matters arising

- Minutes and action agreed as a true record. No matters arising.

3. Communication with the Commission

- All deliverables reported on time no outstanding issues.

4. Contractual

- All partners had signed the Material Transfer agreement with Barcelona. Barcelona had released the SOP
- The material transfer agreement had been set up between the consortium and Washington University in the USA for transfer of the murine norovirus and signed.

5. Expenditure Monitoring

- The bank details of the Spanish Stakeholder was still outstanding. **ACTION:** CS to produce a budget summary including detailed personnel inputs to the project by CSL to date.

6. Legal

- None

7. Ethical

- None

8. Communication with PAB

- Prof Notermans had retired and left the PAB but it was considered that there was no need to recruit another representative as the remaining board were providing comprehensive support⁴.

9. Internal Communication

- Nothing to report
- Partners were urged to respond as soon as possible to other partners, requests.

10. Actions from PAB

- The quality sheet was still outstanding.

11. AOB

- None

12. Date of Next Meeting

- The next CAT meeting will be held on **17th March 2009**.

⁴ Subsequently it was decided to invite two Expert Stakeholders to the PAB.

D5.2

A Tool for Data Analysis

This tool has been located on the project website, on the secure “Beneficiaries” section
<http://www.eurovital.org/secure1/Beneficiaries.htm>

ViTAL Tool for data analysis

Go to:

[Quantification of PDU for water samples](#)

[Quantification of PDU for fertilizer, faeces, serum, liver and meat](#)

This tool can be used to estimate by approximation the number of PCR detectable units (PDUs) per volume of a sample. The estimation is based on the assumption that the PDUs are distributed homogeneously within samples. **If this assumption cannot be made, then this tool cannot be used for estimation of the concentration.** For more accurate estimates, other software tools, such as Mathematica (Wolfram Research, Inc.) can be used. Nevertheless, the results obtained with this tool will provide a good indication of the order of magnitude of virus concentrations in the samples.

This tool works with drop-down menus as well as blank cells that require insertion of volumes or weights. Values that need to be inserted or adjusted are presented in **blue**, whereas automatically calculated parameters are presented in **red**. When entering volumes or weights, please use the numerical pad of the keyboard to assure the correctness of decimal points.

This tool is created for individual samples. Hence, for each sample the spreadsheet has to be completed. The output should be copied into an external file (e.g. Word document or Excel sheet) and the values for the second sample should be inserted. Of course, if the parameter values and results of the molecular analyses are identical between samples, then identical estimators for the concentration will be obtained.

How to complete the spreadsheet?

First confirm (using the pull-down menu) that the assumption of homogeneous mixing is appropriate for the respective virus. Next, complete the parameter-section by entering the appropriate values using the labjournal. Then select the number of (RT-)PCR analyses that were done per sample (*i.e.* in triplicate, then select '3' from the pull-down menu). Subsequently select the number of serial 10-fold dilutions that were examined per repeat, and select from the pull-down menu per dilution the presence ('1') or absence ('0') of target genomes as detected by molecular analyses. The estimated concentration and 95% interval are subsequently provided. Considering the amount of data that has to be processed, this estimation may take some time on slower computers.

Correspondence

In case of any questions regarding this tool or for suggestions for improvement please contact Martijn Bouwknecht.

E-mail: martijn.bouwknecht@rivm.nl

Tel: +31 30 2744290



D7.1 VITAL website

www.eurovital.org



The screenshot shows a Mozilla browser window displaying the VITAL website. The browser's address bar shows the URL <http://www.eurovital.org/>. The website has a yellow background and a navigation menu at the top with links: BENEFICIARIES, EXPERT STAKEHOLDERS, PARTICIPATING INSTITUTES, PUBLIC DELIVERABLES, PRESENTATIONS, WOMEN IN SCIENCE, and LINKS. On the left side, there is a section titled "LAGUNA DESIGN / SCIENCE PHOTO LIBRARY" with a blue, spiky virus-like image. The main content area features the VITAL logo, which consists of the word "VITAL" in a bold, serif font, with the letter "A" replaced by a geometric wireframe structure. Below the logo, the text reads: "Integrated Monitoring and Control of Foodborne Viruses in European Food Supply Chains". The coordinators are listed as: "Coordinator - Nigel Cook", "Assistant Coordinator - Martin D'Agostino" (with "Central Science Laboratory" below), and "Vice Coordinator - Franco Ruggeri" (with "Istituto Superiore di Sanità" below). At the bottom, a paragraph describes the project: "VITAL is a €3.87M EU-supported project which will provide Europe with a framework for monitoring and risk modeling, and prevention of foodborne virus contamination, which will be applicable to any virus, whether existing, emerging or re-emerging, that poses a risk to public health transmitted by food." Below this, a smaller line of text states: "Scientists will use advanced methods for virus detection throughout selected food supply chains from farm to market, to gather data on virus contamination in environmental sources which is suitable for quantitative viral risk assessment. Supply chains will be monitored for the presence of indicator viruses."

*Annex 2 - VITAL Deliverables delivered in the second year up to October 31st
2009*

D5.1 A guidance document on data collection and analysis

D5.3 Document on available models

D1.10 Minutes of 6th CAT meeting

D1.11 Minutes of 4th RDMB meeting

D1.12 Minutes of 2nd PAB meeting

D1.13 Minutes of 7th CAT meeting (not delivered)

D6.1 Optimised cell-culture based propagation methods for HEV and norovirus (not yet delivered)

D1.14 Minutes of 5th RDMB meeting

D1.15 Minutes of 8th CAT meeting

D1.16 Minutes of 9th CAT meeting

D5.1 A guidance document on data collection and analysis

Draft guidance document on data collection in ViTAL

Identification of sampling points

In ViTAL the infection risks for humans by consumption of soft fruits, salad vegetables, pork and shellfish will be estimated by using quantitative viral risk assessment (QVRA). The outcome of the QVRA depends on the value of the data included, and therefore depends on the accuracy and certainty of the estimates. The current sampling plan was developed to benefit most from the fixed number of samples as outlined in Table 1. The strategy includes increasing the probability of detecting virus at a sampling point when low virus concentrations are expected (e.g. irrigation waters) or aiming for most accurate estimates of e.g. prevalence given the total sample numbers. The sampling points were derived from the background review questionnaires that were completed as part of ViTAL's WP6 (Table 2).

Table 1. Total sample sizes per beneficiary after correction of the 15% sample size reduction.

Phase	Food item			
	Soft Fruits	Salad vegetables	Pork ¹	Shellfish
Production	102	102	102	0
Processing	77	77	77	0
Point of sale	51	51	51	51
Total	230	230	230	51

¹ For Defra (VLA), the numbers are 77 for production, 39 for processing and 51 for point of sale, mounting to a total of 167 samples.

Table 2. Overview of the sampling points for the three food production chains. The one sampling point for shellfish is retail and is therefore not included in this table.

Phase	Soft fruits	Salad Vegetables	Pork
Production	Irrigation/surface water	Irrigation/surface water	Blood at slaughter
	Harvester's hands	Harvester's hands	Liver at slaughter
	Harvester's latrines	Harvester's latrines	
	Latrine doorhandle	Latrine doorhandle	
	Surrounding pigs or cattle	Surrounding pigs or cattle	
Processing	None	Water for washing	Meat mincer
Point of sale	Fresh raspberries		Pâté
	Frozen raspberries		Raw meat

Soft fruits and salad vegetables

Production phase

During production, the contamination of fruits and salad vegetables with NoV and HAV is most likely to originate from humans, either directly or indirectly through irrigation water. The contamination by humans results from virus transfer from food handlers' hands to the product. Therefore, harvesters' hands are a primary source for sampling. Absence of virus on the hands may not mean that hands are no source of contamination. Therefore, the doorhandle from toilets and latrines are sampled additionally as possible source for contamination of hands. Furthermore, to conclude whether harvesters were infected or not, swabs from the latrines or toilets that are used by harvesters will be examined.

Processing

Raspberries are reported in the background review questionnaires returned to KU Leuven to be transported directly to the market shortly after harvest. Hence, there is no processing phase. Therefore there will be no sampling during processing of soft fruits. The samples that were reserved for the processing phase are reallocated to the production phase and point of sale. A common procedure for processing of salad vegetables is rinsing with water. This rinsing can result in contamination of produce when untreated or insufficiently treated water is used. Furthermore, virus from contaminated products can be transferred to clean products during rinsing. Therefore, rinsing water is chosen for sampling.

Point of sale

Soft fruits are sold fresh and frozen and salad vegetables are sold as unprocessed or processed (cut and/or mixed). For soft fruits, sampled end-product therefore will include baskets of fresh fruits and boxes of frozen fruits. For salad vegetables, the end-products are unprocessed crops, such as lettuce heads, and bags of processed vegetables, such as mixed salads.

Pork production

Production phase

Contamination of pork by HEV results primarily from HEV-infections in pigs during the production phase rather than from human contamination during processing. To estimate the foodborne risk of HEV for humans, it is important to sample as close to the point of sale as possible. Therefore, sampling will only be conducted at slaughterhouses and point of sale. Meat is most likely to be contaminated via blood when pigs are viremic. Therefore, blood samples will be collected from pigs at slaughter. Furthermore, the liver is the primary site for replication of HEV and consumed as such or used in processed pork products. Therefore, pork liver is chosen as another sampling point at slaughter.

Point of sale

Most likely candidates for HEV-contamination are products that contain liver, such as pâté, and raw meat. Pork chops are chosen as raw meat samples, because this meat type was contaminated by HEV most frequently during experimental infection. The meat mincer can be a source for cross contamination when uncontaminated meat is minced after mincing HEV-contaminated meat. Therefore this sampling point was chosen for swabbing.

Sample sizes

Soft fruits

Table 3 shows the number of samples to be collected per sampling point for soft fruits. In total, 33 samples are collected from the soft fruits production chain per sampling. Given the total of 230 samples available, then seven samplings can be conducted. These samplings should be planned randomly throughout the production period.

The samples from irrigation water should be collected from the source(s). The samples from surface water should be collected on three different locations from water that surrounds the arable land. These samples will provide information on any run-off from the land and thus on potential contamination of crops.

The five harvesters should be chosen at random, e.g. by numbering each harvester and using one (<7 harvesters) or more (otherwise) dices. One swab should be used to cover the two hand palms and all fingers including the nail dirt. The harvesters should be swabbed just before their lunch.

The 10 baskets of fresh and frozen raspberries each sampled at retail should be obtained as randomly as possible, with only one basket per seller. From each of these baskets, a single sample of 25 g should be drawn. At the next sampling occasion, the same sellers can be approached, because the batch of raspberries sold will be different.

Table 3. Number of samples collected and viruses to be examined in these samples for soft fruits.

Sampling point	No. of samples	No. of samplings	Viruses to be examined*
Irrigation water	3 samples of 10 L	7	HAV, NoV, PAdV [†] , BPyV, HAdV
Surface water	3 samples of 10 L	7	HAV, NoV, PAdV [†] , BPyV, HAdV
Harvester's hands	5 randomly chosen harvesters	7	HAV, NoV, PAdV [†] , BPyV, HAdV
Harvester's latrines	1 swab from all latrines	7	HAV, NoV, HAdV
Latrine doorhandle	1 swab from all doorhandles	7	HAV, NoV, HAdV
Fresh raspberries	10 samples from a basket each	7	PAdV [†] , BPyV, HAdV [‡]
Frozen raspberries	10 samples from a basket each	7	PAdV [†] , BPyV, HAdV [‡]

* HAV: hepatitis A virus; NoV: norovirus; PAdV: porcine adenovirus; BPyV: bovine polyomavirus; HAdV: human adenovirus

[†] if PAdV is detected, then samples are analyzed additionally for the presence of hepatitis E virus

[‡] if HAdV is detected, then samples are analyzed additionally for the presence of NoV and HAV

Salad vegetables

Table 4 shows the number of samples to be collected per sampling point for salad vegetables. In total, 37 samples are collected from the salad vegetable production chain per sampling. Given the total of 230 samples available, then six samplings can be conducted (mounting to 222 samples). The remaining eight samples should be used for fresh and processed salads during one of the samplings. The six sampling occasions should be planned randomly throughout the production period.

The sampling procedures as described for soft fruits are also applicable for salad vegetables for irrigation and surface water, for harvester's hands, harvester's latrines, the latrine doorhandle and the products and the points of sale.

For rinsing water, two samples are to be collected prior to the rinsing of the first batch of a production day. The samples after rinsing should be collected after washing of the same batch.

Table 4. Number of samples collected and viruses to be examined in these samples for salad vegetables.

Sampling point	No. of samples	No. of samplings	Viruses to be examined*
Irrigation water	3 samples of 10 L	6	HAV, NoV, PAdV [†] , BPyV, HAdV
Surface water	3 samples of 10 L	6	HAV, NoV, PAdV [†] , BPyV, HAdV
Harvester's hands	5 randomly chosen harvesters	6	HAV, NoV, PAdV [†] , BPyV, HAdV
Harvester's latrines	1 swab covering all latrines	6	HAV, NoV, HAdV
Latrine doorhandle	1 swab covering all doorhandles	6	HAV, NoV, HAdV
Rinsing water	2 source water samples of 10 L	6	HAV, NoV, PAdV [†] , BPyV, HAdV
	2 samples of 10 L after rinsing	6	HAV, NoV, PAdV [†] , BPyV, HAdV
Fresh lettuce	10 sample of one head each	6	PAdV [†] , BPyV, HAdV [‡]
Processed lettuce	10 sample of one bags each	6	PAdV [†] , BPyV, HAdV [‡]

* HAV: hepatitis A virus; NoV: norovirus; PAdV: porcine adenovirus; BPyV: bovine polyomavirus; HAdV: human adenovirus

[†] if PAdV is detected, then samples are analyzed additionally for the presence of hepatitis E virus

[‡] if HAdV is detected, then samples are analyzed additionally for the presence of NoV and HAV

Pork

Production

At the slaughterhouses, a total of 72 blood samples and liver samples should be collected in pairs, meaning that the pig from which blood is sampled should also be sampled for the liver. The blood and liver samples should be traceable to that pig. The samples at the slaughterhouse should be collected on four sampling days (and thus from four different farms), mounting to a total of 8 blood samples and 18 liver samples per sampling occasion. The sampling numbers prescribed for the meat mincer, liver pâté and pork chops should be collected during each of eight randomly chosen sampling occasions. This plan mounts to 232 samples in total.

All samples should be examined in triplicate by RT-PCR as neat and 10-fold diluted samples. Positive specimens should be retested in duplicate in serial 10-fold dilutions to determine the end-point dilutions (this is the serial dilution that is tested negative in duplicate).

Table 5. Number of samples collected and viruses to be examined in these samples for pork production.

Sampling point	No. of samples	No. of samplings	Viruses to be examined*
Blood at slaughter	18 per farm	4	HEV, PAdV
Liver at slaughter	18 per farm	4	HEV, PAdV
Meat mincer	1 swab	8	HEV, HAdV, PAdV [†]
Pâté	5 different pâtés	8	HEV, HAdV, PAdV [†]
Raw meat	5 samples of pork chop	8	HEV, HAdV, PAdV [†]

* HEV: hepatitis E virus; PAdV: porcine adenovirus; HAdV: human adenovirus

[†] in case HAdV is detected, then samples are examined for NoV and HAV.

Virus detection by (RT-)PCR

The viruses that have to be examined by (RT-)PCR in each of the samples are indicated in the last columns of Tables 3-5. All samples should be tested as neat samples first. Depending on the test results, the following should be done.

In case of a negative test result for the target virus and a positive test result for the internal amplification control (IAC), then the result are sufficient and no further testing is required.

In case of a negative test result for the target virus and a negative test result for the IAC, then the sample should be examined as neat sample and 10-fold diluted sample. If the 10-fold diluted sample shows a positive result for the IAC and a negative result for the target virus, then the results are sufficient and no further testing is required.

In case a neat sample tests positive for the target virus (the IAC signal is of no importance in this case), then this sample should be retested in triplicate with sufficient serial 10-fold dilutions to determine the end-point dilution for each of the triplicates.

Appendix 1: What if...?

...irrigation water is obtained from surface water?

Then all samples ($n=6$) should be collected from surface water

...the water used for rinsing the salad vegetables is the same as the irrigation or surface water?

Then one additional sample should be obtained from fresh lettuce and one from processed lettuce

...no latrines are present?

Then swab the toilets that are used by harvesters and/or food handlers

...less than three harvesters are present?

Then sample each harvester and divide the remaining samples equally among the irrigation and surface water.

...harvesters use gloves for harvesting and/or processing?

Then collect both gloves (right and left hand) at the moment the harvesters intend to dispose the gloves.

...the farm size <250 pigs?

Then still collect 18 samples from the pigs. When the farm size <18 pigs, then collect samples from all pigs and divide the remainder equally over the liver pâté and pork chop samples at one of the samplings.

D5.3 Document on available models

Document on available risk assessment models for viruses

A literature review was conducted in PubMed to find studies that assessed the infection risks for viruses quantitatively. In total, 10 such studies were retrieved (Table 1). These papers are discussed below for their relevance to ViTAL.

The viruses

The viruses selected in the described quantitative risk assessment models range from the relatively larger DNA adenoviruses (Crabtree et al., 1997, van Heerden et al., 2005) to the small RNA enteroviruses (Regli et al., 1991, Mena et al., 2003) and rotaviruses (Regli et al., 1991, Haas et al., 1993, Gerba et al., 1996). The enteroviruses included coxsackieviruses (Mena et al., 2003), polioviruses type 1 and 3, and echovirus 12 (Regli et al., 1991). Some refer to viruses in general (Petterson en Ashbolt, 2001, Petterson et al., 2001, Hamilton et al., 2006). In ViTAL the viruses that need to be studied are norovirus, hepatitis A and E virus, and adenovirus.

The matrices

Only three out of ten papers describe quantitative risk assessment models for crops (Petterson en Ashbolt, 2001, Petterson et al., 2001, Hamilton et al., 2006). One of these three is a methodological study to examine the implications of overdispersion in virus concentration on crops (Petterson en Ashbolt, 2001). Masago et al. (2006) estimate the risk of a norovirus infection due to oyster consumption, but this risk assessment is semi quantitatively and the approaches therefore not of interest to ViTAL. The others estimate risks for drinking and/or recreational waters. In ViTAL the food chains to be modeled in the quantitative risk assessment include soft fruits/salad vegetables, pork meat products and shellfish. Selection of these matrices was based on the different sources of virus, the ways of virus contamination of the foods and the different intervention measures.

The models

Regli et al. (1991) and Haas et al. (1993) provide a theoretical background to assess an infection risk due to consumption of drinking water, which can be useful for other matrices as well. Regli et al. (1991) describes the assumptions that are required to be made in virological risk assessment and evaluates different dose-response models (i.e., exponential vs. beta Poisson). Haas et al. (1993) provides an approach for including uncertainty and variability into risk assessments. The theories about distributions, homogenization, dose-response models and uncertainty and variability described in these two papers are valuable for the QVRA performed for ViTAL.

The three models dealing with food products focus on the use of irrigation water for salad crops (Petterson en Ashbolt, 2001, Petterson et al., 2001, Hamilton et al., 2006). Petterson et

Table 1 Quantitative Risk Assessments for Viruses in the Environment

Matrix	Virus	Title publication	Authors	Reference
Drinking water	Rotavirus, Poliovirus 1 (2x), Poliovirus 3, Echovirus 12	Modelling the risk from Giardia and viruses in drinking water.	Regli S, et al.	J Am Water Works Assoc 1991; 83: 76–84
Drinking water	Rotavirus	Risk assessment of virus in drinking water.	Haas CN, et al.	Risk Anal 1993; 13(5): 545–552
Drinking and recreational waters	Rotavirus	Waterborne rotavirus: a risk assessment.	Gerba CP, et al.	Water Res 1996; 30: 2929–2940
Drinking and recreational waters	Adenovirus	Waterborne adenovirus: a risk assessment.	Crabtree KD, et al.	Water Sci Technol 1997; 35: 1–6
Salad crops	Enteroviruses	Viral risks associated with wastewater reuse: modeling virus persistence on wastewater irrigated salad crops	Petterson and Ashbolt	Water Sci Technol 2001; Vol 43 No 12 pp 23–26
Salad crops	Viruses	Microbial Risks from Wastewater Irrigation of Salad Crops: A screening - Level Risk Assessment	Petterson et al.	Water Environ Res 2001; 72 (6): 667-672
Drinking and recreational waters	Coxsackie virus	Risk assessment of waterborne coxsackie virus.	Mena KD, et al.	J Am Water Works Assoc 2003; 95: 122–131
Drinking water; Recreational water	Adenovirus	Risk assessment of adenoviruses detected in treated drinking water and recreational water.	Van Heerden J, et al.	J Appl Microbiol 2005; 99(4): 926–933.
Raw Vegetables	Viruses	Quantitative Microbial Risk Assessment Models for Consumption of Raw Vegetables Irrigated with Reclaimed Water	Hamilton et al.	Appl Environ Microbiol 2006; 72 (5): 3284–3290
Drinking water	Norovirus	Quantitative Risk Assessment of Noroviruses in Drinking Water Based on Qualitative Data in Japan	Masago et al.	Environ. Sci. Technol. 2006; 40: 7428-7433

al. (2001) model the clinging of viruses to lettuce crops through sprayed irrigation water. However, the volume of retained water was obtained from a study in which lettuce heads were completely immersed in water (Shuval et al., 1997). This procedure may not represent the volume of water retained after irrigation, which therefore should be examined. Furthermore, actual consumption data were not included in the model, but instead the risk was verified for a fixed consumption of 100 g. Hamilton et al. (2006) estimated the risk of infection for enterovirus due to consumption of arable crops, including lettuce, that were contaminated by irrigation water. The authors

conducted field experiments to estimate the amount of irrigation water retained on broccoli and cabbage, and used the previously described estimates from Shuval et al. (1997) for lettuce. The remainder of the study is similar to that of Petterson et al. (2001), with the difference that consumption is represented as function of bodyweight by Hamilton et al. (2006). The experimental data on water retention on broccoli and cabbage may be useful for VITAL for assessing the risk posed by irrigation water.

Health based targets

The risk for public health in the described studies is presented as probability of infection, sometimes translated into probability of disease or mortality by using point estimates for morbidity or mortality from outbreaks. Another measure to represent possible adverse health effects for humans is an estimate of the disability adjusted life years (DALY). For instance, the WHO uses disability adjusted life years (DALY) to measure the global burden of disease (Lopez et al., 2006). Furthermore, targets for public health protection set by WHO are based on DALYs rather than on infection risks. A DALY consists of the years of life lost due to premature mortality and the period of time spent in a suboptimal health status as a consequence of infection. Thus, a DALY estimate includes the translation from infection to actual disease and may give better insight into the adverse health effects of pathogens in the environment than an infection risk (infection does not necessarily produce illness). However, the translation from infection to disease is highly variable and depends on multiple host and pathogen characteristics. The available information for NoV, HAV and HEV is insufficient to estimate robustly the incidence of disease and associated health effects within VITAL. Therefore, the QVRA for ViTAL will provide estimates of exposure of humans to the viruses through the mentioned food products and not estimates of DALYs.

Data gaps

Irrigation water is one of the sampling points in ViTAL (Deliverable D5.1: A guidance document on data collection). Thus, the process of virus contamination due to irrigation will be modeled. To estimate the concentration of virus on products it will be important to assess the volume of retained water on raspberries and lettuce heads as a function of the duration of irrigation. Furthermore, the clinging of viruses to food products and wash-off during prolonged irrigation need to be determined. The currently available data for lettuce is insufficiently accurate for use. For raspberries, no data are available.

In addition, the inactivation rates of NoV, HAV and HEV need to be examined during their presence on the surfaces of food products and during storage. For HEV, inactivation during processing of raw meat (e.g., by fermentation) need to be assessed.

Furthermore, rinsing of salad vegetables and soft fruits may result in cross contamination. The attachment and detachment rate of viruses during rinsing is currently unknown. This process will be important for modeling the fate of the viruses during the production phase.

Consumption data are required to estimate the doses that are ingested by people. Furthermore, the state of the food product at consumption needs to be known, in order to account for risk reducing effects due to preparation of the food.

Dose-response models are not available for HAV and HEV. For Norovirus, a dose response model is described for Norwalk virus, the prototype norovirus (Teunis et al., 2008). The absence of an adequate dose response model for HAV and HEV may be addressed by adopting the rotavirus dose-response model. Rotavirus has a high infectivity (i.e., the probability of infection per virus particle), and using this dose response model likely prevents the infection risk from being underestimated.

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D1.10 Minutes of 6th CAT meeting

6th Core Administration Team Meeting

17th March 2009

Minutes and Actions

Attendees:

Nigel Cook (NC)
Christina Steveni (CS)
Martin D'Agostino (MDA)

2.1 *Item*

1. Apologies for absence

- None

2. Minutes of the last meeting and matters arising

- Minutes and action agreed as a true record. No matters arising.

3. Communication with the Commission

- All deliverables reported on time no outstanding issues.

4. Contractual

- It had come to light that a group working with the WHO were producing a code of good practice which is a key deliverable in the VITAL project. The coordinator was keen to explore ways of working in cooperation with this group.

2.2 5. *Expenditure Monitoring*

- The bank details of the Spanish Stakeholder was still outstanding. **ACTION:** CS to produce a budget summary including detailed personnel inputs to the project by CSL to date.

6. Legal

- None

7. Ethical

- None

8. Communication with PAB

- Two new stakeholders had been invited to join the PAB: Clive Thompson and Luc Peeters.

9. Internal Communication

- Nothing to report
-

10. Actions from PAB

- The quality sheet was still outstanding.

11. AOB

- None

12. Date of Next Meeting

To be decided.

D1.11 Minutes of 4th RDMB meeting

4th Research and Dissemination Management Board Meeting

**6th and 7th April 2009
Hotel Park, Novi Sad, Serbia**

Those present: -

Defra (FERA) N. Cook, M. D'Agostino, A. Gilbert, C. Steveni
Defra (VLA) – M. Banks
KULeuven – K. Willems
VRI – P. Vasickova
UH – L. Maunula
UPA – A. Vantarakis
ISS – F. Ruggeri
RIVM – A. De Roda Husman
WUR – W. van der Poel
NVRI – A. Rzezutka
NIV-NS – , S. Lazic, T. Petrovic
UL-BF – P. Raspor
ITACyL – D. Rodriguez-Lazaro
UB – R. Girones

Plus the Project Advisory Board

Dr Peter Wyn-Jones (UWA)

*Prof. K. Clive Thompson (ALcontrol Laboratories, Secretary)**

Dr Jan Vinjé (CDC)

Dr Luc Peeters, (MV)

Dr Fleming Hansen, Danish Meat

During 1st session: Dr. Laurence Moreau, EC DG Research, Project Officer

1. Consortium and MTA points (Day 1, Consortium Meeting Agenda item 17:30 – 18:30)

By way of an informal review of the formalities, Dr Gilbert reviewed clauses relating to confidentiality in the Consortium Agreement (at 11) and Grant Agreement (at 11.9) and explored where and how the Beneficiary partners should beware of potential for conflict of interest. For the Commission, Dr Moreau confirmed that the VITAL foreground IP would be necessary to protect pending final delivery of the contract and publication of the results and other outputs. However, she was content to leave management of this to the discretion of the Co-ordinator / Core Administration Team.

It was agreed that from this point forward (and marked by the re-issue of SOPs in relation to the need to include an extraction blank) all VITAL SOPs would be designated and marked 'In Confidence'. Similarly, all VITAL data and other draft outputs (e.g. Code of Practice) would be so designated and marked. Other items may follow, and, for the avoidance of doubt, the

secure VITAL website would henceforth indicate the confidentiality status of project materials. It was recognised that the need to conduct concurrent (but separate) science by Beneficiaries, as well as the demand for Ph.D. students to publish papers in a timely way during the course of VITAL, had to be considered. Nigel Cook confirmed that he was always available to advise in case Beneficiaries had doubts about how they might proceed in any instance.

It was anticipated that formal ‘confidential’ status would be in place for methodology by the completion of all pre-collaboration ring trials, and certainly during the conduct of full ring trials.

Dr. Moreau informed that she would contact the European Commission’s Codex Contact Point, with regard to them inviting the Coordinator to participate in the Working Group Viruses, representing the VITAL project. This would be a step to try to harmonise the development of Codex’s Code of Hygienic Practice for Control of Viruses in Food, with the VITAL COGP while ensuring that confidentiality is maintained.

2. News from the CAT

The VITAL Annual Report is due to the Project Officer on May 15th.

3. Project progress

Progress of VITAL was discussed at the current Consortium Meeting, and will appear in annual report.

4. Financial Matters

The Form Cs should be completed by each Beneficiary, and sent to Christina Steveni by April 30th. Any request for transfer of funds between categories should be made in the first instance to the Coordinator. Martin D’Agostino will put all financial information and relevant links on the VITAL website.

5. Plan for 1st Annual Report delivery

All WP leaders are to submit a report of the 1st year’s activities to the Coordinator, by April 30th.

6. A.O.B.

VITAL Women in Science Prize

It was agreed that each participating organisation will donate 150 Euro for a prize for the VITAL Women in Science Prize. The prizewinner will be selected by nomination to the Project Advisory Board in the final months of the project. The prize will be 1500 euro and the winner will receive a trophy / certificate and give a special lecture at the final meeting.

Net Viewer

The Netviewer facility has now been available for one year, but it has very seldom been used for anything other than the RDMB meetings. This facility was purchased for all the Workpackage Leaders as a tool for communicating to their WP participants. So far however there has only been one instance of this type of use. WP leaders are urged to make full use of this facility henceforward, to regularly communicate with their participants. Please ensure that all meetings are entered into the Meeting Planner in the Netviewer facility. If anyone needs refresher training on using Netviewer, they should please contact Martin D'Agostino.

Ethical issues

Dr. Gilbert responded to points that had arisen in prior discussion of the approach to: human sampling (hand swabs) and simulation of the fact-finding missions processes.

The meeting confirmed that the Project proposal (at B4 ETHICAL ISSUES, p74) stated "The project does not raise any ethical issues.". This being the case, it is imperative that practical investigations are conducted strictly so as to avoid raising any such issues, which, should they arise, could otherwise not be evaded afterwards.

Therefore, the following policy must be followed without exception:

Human hands must only be sampled anonymously, and results treated in a fashion and coded so as to keep results 'blind' as to directly connecting any result with the volunteer subject's identity.

Visits to inspect premises by fact-finding missions should be described as 'mock audits' (or something similar) to clearly distinguish this VITAL practical exercise from any actual audits, which would be conducted on a different formal basis (as to relationships between the parties involved), albeit using identical standard methodology. VITAL WP6 fact-finding missions should approach each mock audit 'inspection' expecting to take things as they find them. However, in the foreseeable (if unlikely) event that, in the course of an inspection, a VITAL fact-finding mission should encounter a situation that they would view as an infringement of good hygienic practice of such significance that it could present an unacceptable risk to public health, they must:

- cease their inspection, suspend the exercise and explain to the proprietor of the premises their reasons for doing so;
- contact Andrew Gilbert (cc. Nigel Cook) giving a brief description of their concerns and enquire whether this "Exceptional Event" may constitute a *force majeure*; sufficient to stop (at least) this part of the Project;
- at your discretion, and following the passage of time during which the above question was addressed, you may consider resuming the inspection. Your reasons

for this decision may reflect factors such as the proprietor's reaction upon hearing of your original concerns, and their subsequent response on the ground, among other matters you may consider to be relevant.

- unless the VITAL WP6 fact-finding missions feel confident that they can complete any inspection without placing themselves in a situation of 'guilty knowledge' (from which ethical issues must arise), they must discontinue that part of the study and consult with Nigel Cook as soon as possible as to the way forward.

On no account must any VITAL project data or findings be communicated to any individual subject or provider of premises, or any promise made to do so whether in return for consideration (e.g. co-operation with the VITAL study) or not, without the express permission of the Coordinator.

D1.12 Minutes of 2nd PAB meeting

VITAL

PROJECT ADVISORY BOARD 2

APRIL 7TH 2009

Scientific Veterinary Institute "Novi Sad", Serbia

MINUTES

Present Dr Peter Wyn-Jones (University of Aberystwyth, Chair)*, Dr Nigel Cook (FERA, VITAL Co-ordinator), Mr Martin D'Agostino (FERA, observer), Dr Fleming Hansen[†] (Danish Meat Research Institute), Dr Franco Ruggeri (ISS, Vice-Co-ordinator), Mr Luc Peeters[†] (Mechelen Auctions), Prof Clive Thomson* (ALcontrol), Dr Jan Vinjé* (CDC).
(*external member, [†]stakeholder representative)

13. Welcome and Introductions

13.1. The Chairman welcomed members to the second meeting of the Board, and introduced Mr Peeters and Mr Hansen, who had replaced Dr Notermans and Dr Hugas. The Chairman reminded members that the PAB would review progress, act as a point of contact for the Project team and be available to advise in case of technical or managerial difficulties. The Board is not acting on behalf of the Commission.

14. Agenda

14.1. The Meeting focused on issues from (a) the first PAB Meeting, (b) a briefing paper sent in advance to all PAB members by the Co-ordinator, and (c) the current VITAL Consortium Meeting.

15. Issues arising

15.1. Quality Management System (QMS). There is still no documented QMS for the Project nor is there an individual acting as Quality Manager. It would be especially important to have a QMS in place should the Commission undertake a technical audit at any time. **NC agreed** that a QMS would be set up covering all aspects of the Project. A QMS is also necessary to ensure consistency of approach in familiarisation with the SOPs and competency assessment (see 3.3). Implementation of such a system was not discussed.

15.2. Reference materials. It was reported to the PAB that pre-collaborative ring trials are in progress to demonstrate the use of SOPs. Two trials have been conducted and two more are planned. It was suggested that viral nucleic acid samples could be circulated as reference materials to assist laboratories in gaining competence in virus detection procedures.

15.3. Competency assessment. Training and familiarisation in the SOPs had taken much longer than originally planned. It was recognised by the Board that much effort had

gone into the refinement of SOPs for sample processing and viruses detection, and those completed are now on the Project's website. However, even after a year all are still not finalised. The time to achieve individual laboratories' competency in the methods had been extended by revision of the DoW (with Commission agreement). This strategy was supported by the PAB as competence in this area is critical. It was unclear how laboratories will demonstrate their competence in the SOPs. To recover lost time WPs 2-4 will now run concurrently rather than consecutively. The PAB sought assurances from the Project team that this would not affect the programme adversely.

15.4. Virus detection. Much time on development of QPCRs has already been expended. If the QPCR technique is not used, then significant project expenditure will have been wasted. As an urgent priority an agreed way forward should be agreed and documented. **JV agreed** to provide advice on converting QPCR data to MPN results. Consideration should be given to combining (e.g.) three samples with pooling of the nucleic acid as a way to reduce consumable costs and allow more samples to be analysed.

15.5. Site fact-finding missions and sampling.

15.5.1. The PAB expressed concern that there was a manifest lack of effective communication between the 'fact-finding missions' and 'laboratory' (data-gathering) teams. This needs to be addressed urgently. The purpose of the site fact-finding missions should be clarified and agreed. It was pointed out that, in this project, site fact-finding missions are information-gathering exercises to enable proper sampling and analysis procedures to be implemented, and they are not being done for accreditation purposes. The plan to combine fact-finding missions with (some) sampling was recognised as well-intentioned, but in the light of experience it may now be better to separate the two activities while at the same time ensuring that the two teams demonstrate a common thought-through purpose to the 'client'. Rationalisation of the sites/matrices has helped in the resolution of this problem but much remains to be done.

15.5.2. Input from an agronomist should be considered.

15.5.3. The PAB felt that there are too many pre-farm gate samples. **LP offered to provide guidance** on this issue.

15.5.4. Consideration should be given to producing an agreed documented sampling protocol to avoid inconsistent data being generated. Lack of cohesion on sampling issues could be serious.

15.5.5. Sampling of imported products to be retained but clearly defined (= outside of EU) and sampled at point of entry rather than point of sale to avoid any potential contamination after entry.

15.5.6. An effective QMS would include these issues.

15.5.7. Several other concerns were raised in connection with sampling, but were addressed later in the Consortium Meeting Data-Gathering Workshop (Drs Rutjes and Bouwknecht).

15.6. Project Management. RDMB meetings have been held on schedule using the Net Viewer facility and these have gone well, though the Co-ordinator reported that it may be better to hold one face-to face meeting per year. He expressed some concern that deadlines for submission of various reports etc had not been met and sought

guidance from the PAB. It was suggested that withholding payment should be regarded as a 'last-resort' measure and that clarification of the implications that non-compliance by the defaulter would cause to the whole project is a better way to proceed.

- 15.7. Final Conference. The Board felt that the very ambitious event proposed to run in May 2011 in Ljubljana needs urgent consideration now. An organising committee should be set up to agree the conference programme areas; the length and dates of the event and this information should be publicised as soon as possible.

In conclusion, the Chairman, on behalf of the PAB, congratulated the Project Team on the progress made so far and on tackling some of the difficult issues associated with the Project.

PWJ April 09

D1.14 Minutes of 5th RDMB meeting

Research and Dissemination Management Board Meeting Held by Netviewer on 29th July 2009

Present:

FERA

VLA

KULeuven

VRI

UH

UPA

ISS

RIVM

NVRI

NIV-NS

BH-UL

ITACyL

1. News from the CAT

Partners were reminded that the delivery date of the VITAL Deliverables refers to the date when they should be delivered *to the Commission, not to the Coordinator*. Partners responsible should therefore please send the Deliverables to the Coordinator at least 1 week before the due date, e.g. if the delivery date is 31st October, then the Deliverable should be sent to the Coordinator at least by the 24th October.

The Form Cs will be sent out in due course for signature. We are awaiting the resolution of a problem with the system for generating the forms, and as soon as this is sorted the forms will be sent.

The document “Dissemination Activities in VITAL”, which has been distributed to all Beneficiaries, stipulates the procedure which must be followed henceforward for all publications / presentations etc. from VITAL. All partners agreed to the contents of the document. It will be placed on the website.

The VITAL Quality checksheet will be prepared and sent out shortly.

2. Ring Trial news

All results except for UPA have now been received. David Rodriguez-Lazaro is currently at FERA, analysing the results with Martin D’Agostino. Once they have a global picture of the results for all partners, they will check to see if there are any specific problems with controls etc. NVRI and VRI reported issues with the SPC virus, but it was agreed that this would be

discussed in a separate Netviewer meeting once all results have been analysed. If any other issues arise a Netviewer meeting will be set up to discuss them with all relevant partners.

3. Progress of Workpackage 2 – 6

The VITAL sampling strategy was discussed. The documents “Notes on the VITAL Sampling Strategy”, and “Decisions of WP5 / WP6 meeting”, were put on the screen for reference. The latter document was slightly revised to clarify that the six samples in points 3 and 6 are ad hoc samples. The information contained in both documents will be merged to finalise the strategy, and then placed on the website.

Kris asked for all data-gathering partners to give at least a 2-week window for the fact-finding visits. Also, plenty of notice before asking him and the fact-finding team to visit is necessary, due to the need to keep the flight costs as reasonable as possible.

A list of completed background review questionnaires, fact-finding visits performed, and visits planned (with dates) will be placed on a Fact-Finding Missions section of the project website. This section will also contain reports of each visit, with areas of interest described and numbers of samples taken given.

Data-gathering partners were urged to complete the background review questionnaires as soon as possible.

Martijn Bouwknecht will be preparing and sending individualised Sampling Guidance Documents for each phase and each partner, when the relevant background review questionnaires have been received and analysed by KULeuven and RIVM.

Any individual issues over timing of sampling and / or numbers of samples which should realistically be taken in each year, should be discussed on an individual basis between each partner and RIVM.

If the owners of premises from which samples are taken wish for some feedback after the fact-finding visit, it can be given to them on the strict understanding that the feedback is informal, and not related to the VITAL project.

It was re-emphasised that when samples are taken from individuals, i.e. the harvesters' hands samples, they must be taken anonymously and under no circumstances should names be recorded.

4. VITAL Symposium planning and contingency planning

The agenda of the Symposium was discussed. The draft agenda was agreed, but it was recognised that small changes could be made later, e.g. if one group has very significant results.

The timing of the meeting was extensively discussed. Some partners felt that as much time as possible should be allowed for analysis of all data before presenting the VITAL work. The Coordinator agreed to informally discuss with the Project Officer a possible extension of VITAL to June 2011, to clarify this point of debate finally.

The final date, the venue in Ljubljana, and the first notification of the Workshop and Training Courses will be ready in October 2009. Partners were requested to contact their national regulatory bodies and industrial contacts to advertise the meeting. Franco Ruggeri offered to contact colleagues in EFSA regarding it.

5. Financial Matters

None discussed

6. Possibility of face-to-face RDMB

Partners agreed that a face-to-face meeting would be very useful. Franco Ruggeri kindly offered to host one in Rome in late October / November 2009. Partners were asked to send their availability to the Coordinator for this time; also and very importantly whether they had funding available in their VITAL travel budget to allow travel to such a meeting this year.

7. Communication issues

Partners were once again reminded to please use Netviewer as much as possible.

8. Dates of Year 2 RDMBs

These will take place during the months specified in the DoW. The exact dates will be sent out soon from the CAT

D1.15 Minutes of 8th CAT meeting

8th CAT Meeting
17th June 2009

Minutes and Actions

Attendees:

Nigel Cook (NC)
Christina Steveni (CS)
Martin D'Agostino (MDA)
Andrew Gilbert (AG)

2.1 *Item*

1. Apologies for absence

- None

2. Minutes of the last meeting and matters arising

- Minutes and action agreed as a true record. No matters arising.

3. Communication with the Commission

- A couple of deliverables were delivered slightly late to the Commission (D5.1 and D5.3) To avoid this problem it was decided to make it clear to participants that the delivery date in the DoW was the date the deliverable needed to be delivered to the Commission and the Coordinator needed it at least one week earlier to meet this deadline.
- There was a problem with Partner 11's cost model CS was in discussion with the Commission to resolve the issue.
- The first year report was delivered a week late to the Commission NC to set deadline of 3 weeks for completion of partners contribution to the second year report.

4. Contractual

- A letter would be sent to partners to clarify the procedure for obtaining authorisation for under taking dissemination activities and charging these costs to VITAL (see attached letter).

2.2 *5. Expenditure Monitoring*

- CS would authorise the 20% payments retained from the advance to partners shortly.

6. Legal

- None

7. Ethical

- None

8. Communication with PAB

- The report from the PAB was delivered on time.

9. Internal Communication

- Nothing to report
-

10. Actions from PAB

- The quality sheet was still nearly completed.

11. AOB

- The venue for the final conference would be discussed at the RDMB on the 29th July.

12. Date of Next Meeting

The next CAT meeting (D1.16) will be held on 24th September 2009

D1.16 Minutes of 9th CAT meeting

D1.16 VITAL
9th CAT Meeting
24th September 2009

Minutes and Actions

Attendees:

Nigel Cook (NC)
Christina Steveni (CS)
Martin D'Agostino (MDA)
Andrew Gilbert (AG)

2.3 *Item*

1. Apologies for absence

- None

2. Minutes of the last meeting and matters arising

- Minutes and action agreed as a true record. No matters arising.

3. Communication with the Commission

- Deliverable 6.1 was very late – it was due on the end of July. The project officer had expressed concern over the delay and asked for an explanation. The deliverable was sent on 28th August but no explanation was provided.
- The first year report was delivered to the Commission who had come back with some comments that needed addressing. These were being addressed.
- A mid term review was necessary but this did not fall naturally into the project meeting schedule. The scientific officer would be contacted to see if it was possible to delay the review until the next project meeting. ACTION: Nigel Cook to contact Laurence Moreau.

4. Contractual

- Form C. These had been sent to the commission.

2.4 5. *Expenditure Monitoring*

- No action required

6. Legal

- It was decided that Fera would meet the cost of the materials prepared by Yorkshire Biosciences for ITACYL.

7. Ethical

- None

8. Communication with PAB

- The PAB would be asked if they could suggest external reviewers for the mid term project review.

9. Internal Communication

- Nigel Cook had been invited by DG SANCO to attend the next meeting of the WHO code of good practice.
- Nigel Cook to liaise with Peter Raspor and Kris Willems.

10. Actions from PAB

- The quality sheet was completed.

11. AOB

- The venue for the final conference was set for Ljubljana in March.

12. Date of Next Meeting

- The next CAT meeting (D1.17) will be held on 25th November 2009