Executive summary:

The concept of VITAL is the integrated risk assessment and management of contamination of the European farm to market food chain by pathogenic viruses. The VITAL consortium was composed of expert practitioners in food analysis, quantitative viral risk assessment (QVRA), risk management, and consumer safety. Together, their vision was an integrated approach to the management of foodborne viruses in Europe.

VITAL developed new methods, or adapted and modified existing ones, to produce a portfolio of standard operating procedures to mediate the effective monitoring of four food supply chains - salad vegetable, soft fruit, pork, and shellfish. The first three supply chains were monitored in their production, processing and point of sale phases, whilst the shellfish supply chain was monitored only at point of sale. The principal viruses which were monitored were norovirus, hepatitis A virus, and hepatitis E virus. VITAL also monitored for viruses (human adenovirus, porcine adenovirus, bovine polyomavirus) which would indicate that a route of contamination existed from humans or animals to the food supply chain. Using these methods, an extensive amount of data on virus prevalence was collected, which revealed vulnerability to virus contamination at several points in each food supply chain. Using the data, risk assessments were performed, which have shown that estimated health risks were significant in some cases (e.g., NoV in shellfish or HEV in pork sausage) when consumption and dose-response were considered in combination with the data on virus concentrations in different sources and foods along the food production chains.

VITAL performed a series of fact-finding missions to examine the food safety management practices in the supply chains where the data on virus contamination was gathered. The information acquired through these missions showed that key areas of concern were non-compliance with good prerequisite safety management practices that could open vulnerabilities in the food supply chains to virus contamination. Notably, in primary production of soft fruit and salad vegetables, analysis of areas of concern and virus contamination data revealed correlation between key non-compliances (poor quality irrigation water, poor sanitation, poor hand hygiene) and contamination of produce. VITAL has determined that in particular that compliance with prerequisite programs, such as the forthcoming Codex Guidelines, is essential to reduce the risk of contamination of food supply chains with viruses. To complement the Codex guidelines, and assist in compliance with prerequisite safety programs, VITAL Guidance Sheets were developed. With clear recommendations on regaining control through compliance with prerequisite programs, and the monitoring procedures which VITAL has outlined, the aim of integrated monitoring and control of foodborne viruses in the food supply chains can be fulfilled.

Project Context and Objectives:
The concept of VITAL is the integrated risk assessment and management of contamination of the European farm to market food chain by pathogenic viruses. The VITAL consortium was composed of expert practitioners in food analysis, quantitative viral risk assessment (QVRA), risk management, and consumer safety. Together, their vision was an integrated, multidisciplinary approach to the management of foodborne viruses in Europe.

Members of the VITAL consortium were participants in two European Networks: COST Action 929 "European Network for Food and Environmental Virology" (see http://www.cost929-environet.org online), and the Network of Excellence MedVetNet (see http://www.medvetnet.org online), specifically Work Package 31 "ZOOVIR-NET". The project drew together common aims of each network. These Networks agreed that a major issue regarding foodborne viruses is the lack of effective risk management strategies and prevention and intervention measures against food and environmental contamination. 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. VITAL, focused on the production and processing phases, with the aim of moving away from the concept of endpoint monitoring towards verification monitoring. VITAL's aim was to achieve the following core scientific and technological objectives:

Objective 1: To acquire data on virus contamination of food and environmental sources
VITAL planned to use standardised detection methods to detect norovirus, hepatitis A virus and hepatitis E virus, and representative enteric (index) viruses (human adenovirus, porcine adenovirus, and bovine polyomavirus) throughout three food supply chains – salad vegetable, soft fruit, and pork - from farm to market (and also at point-of-sale for shellfish). Eight European data-gathering laboratories in eight countries used 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. In the different product groups, the presence of indicator viruses commonly found in case of faecal contamination events were considered suitable to distinguish between virus strains of human and animal origin, to indicate whether the points at which samples were obtained would be open to general virus contamination from a specific source.

Objective 2: To assess foodborne viral risks for determining high risk situations and efficacy of interventions
VITAL aimed to develop modelling tools to analyse the data on virus contamination collected at each stage (i.e. production and processing) of each food supply chain to estimate the quantitative viral risk for each scenario studied. The project would carry out sensitivity analyses to reveal the parameters most strongly influencing the risk. Furthermore, VITAL aimed to design Quantitative Viral Risk Assessment models so that rolling revision to assess efficacy of intervention measures could be undertaken.

Objective 3: To develop new measures to prevent virus contamination of foods and the environment

VITAL intended to use the data from monitoring of raw materials and food processing with Modular Process Risk Models (MPRMs) to build up specific hazard analysis critical control point (HACCP) recommendations. The project would take into account and harmonise recent developments in risk management such as the Codex Alimentarius Commission Guidelines on the Application of General Principles of Food Hygiene to the Control of Viruses in Food to aim towards the reduction of foodborne viral infections.

Objective 4: To develop and assess measures for virus reduction and control in case of virus contamination

VITAL aimed to augment the body of information on survival of viruses in foods, and on the effect on viruses of disinfection procedures used in the food industry, to help to elucidate the critical points where viruses may be controlled within the food chain. This objective also required an examination of the effectiveness of vaccination to control hepatitis E virus in pigs.

VITAL desired that its outcomes must be of value to Europe, and to this end must be communicated effectively. To ensure this, a full and targeted dissemination plan would be prepared. VITAL would consolidate and deliver its findings by publishing industry- and practitioner-directed guidance on appropriate control practices for virus contamination, and by presenting to government policy-makers and regulatory agencies the requirements necessary for establishing reliable monitoring of food chains for viruses on a regular or as-needed basis.

Ultimately VITAL aimed to provide to Europe a framework for monitoring, for risk modelling, and for procedures for the control of foodborne virus contamination, which will be applicable to any virus that poses the danger of being transmitted by food. Implementation of such a framework of preventive or proactive virus contamination management should form a first line of defence against transmission of foodborne viral diseases in Europe.

Project Results:

Viruses and food supply chains

Pathogenic viruses originate from two sources - humans and animals - to contaminate the food chain. The World Health Organization /Food and Agriculture Organisation listed noroviruses (NoV) and hepatitis A viruses (HAV) as priority foodborne virus hazards (WHO, 2008), and shellfish, soft fruit and salad vegetables as the food commodities most at risk of contamination with these agents. The strains of norovirus (NoV) which infect humans, and HAV, originate from humans themselves; there is no known animal source for these disease agents. Hepatitis E virus (HEV) appears endemic in pig herds, and therefore WHO / FAO also identified this virus as a significant emerging hazard, with consumption of contaminated pork products conferring a risk of transmission.

VITAL aimed to identify the points in food supply chains at which contamination with viruses could be monitored. To facilitate identification of the origin of contamination, whether human or zoonotic, VITAL looked for the presence of human and animal viruses at various points in the food supply chains. NoV, HAV and HEV were sought, but as it was considered that the prevalence of these significant pathogenic viruses in supply chains such as those for soft fruit and salad vegetables may not be high, or at least not consistently high, it was proposed also to look for other enteric viruses which if they were found would reveal that a route of contamination existed from source to sampling point. Adenoviruses infect both humans and a wide variety of animal species, are shed in large numbers in the faeces of infected individuals (Granoff and Webster, 1999), and are capable of robust survival (Cook and Rzezutka, 2006). They have been proposed as an index of viral contamination, and the specific detection of adenoviruses from human or animal origin is a useful tool for tracing the source of faecal viral contamination (Maluquer de Motes et al., 2004; Wyn-Jones et al., 2011). Hundesa et al. (2006) stated that due to their higher prevalence in fecal and environmental samples than bovine adenoviruses, bovine polyomaviruses are better candidates for tracing a bovine source of viral contamination (a possible hazard regarding animal rotaviruses, see Cook et al., 2004) . Human and porcine adenoviruses, and bovine polyomavirus, will thus be defined as "index" viruses in VITAL.

	Samples analysed for					
	HAdV	BPyV	PAdV	HAV	NV	HEV
Production chain	Soft fruit	?	?*	?*	?	?	?
	Salad vegetables	?	?*	?*	?	?	?
	Pork products	-	-	?	-	-	?
	Shellfish	?	?	?	?	?	?

HAdV: human adenovirus; BPyV: bovine polyomavirus; PAdV: porcine adenovirus; HAV: hepatitis A virus; NV: norovirus; HEV: hepatitis E virus
?: in each sample (* but not latrine samples or harvesters' hand washings).
?: only if presence indicated (see above).
-: not taken

Detection methods for viruses in food supply chain samples

The outcomes of VITAL depended heavily upon the availability of effective methods to detect viruses in the several sample types that would be taken

in the four food supply chains under study. An intensive program of method development was undertaken within the project, to develop new methods or refine existing methods so that they would be fit for purpose. A set of standard operating procedures (SOPs) was prepared for use by the laboratories that would analyse samples and gather information on virus contamination. VITAL used real-time polymerase chain reaction (qPCR) and reverse transcription qPCR to detect the target viruses, and employed various procedures to extract the target viruses from the sample matrices. VITAL promoted the use of a complete suite of quality controls (D'Agostino et al. 2011), including sample process controls (SPC; Diez-Valcarce et al., 2011a) and internal amplification controls (IAC; Diez-Valcarce et al., 2011b) to ensure the correct interpretation of the results. The protocols which were used within the project are listed in Table 2, stating which were developed entirely by VITAL or modified from previously published procedures.

Protocol Source VITAL modification
Human adenovirus qPCR assay Hernroth et al. (2002) Incorporation of IAC (Diez-Valcarce, 2011b)
Porcine adenovirus qPCR assay Hundesa et al. (2009)
Bovine polyomavirus qPCR assay Hundesa et al. (2010)
Norovirus ggI reverse transcription qPCR assay Svraka et al. (2007)
Norovirus ggII reverse transcription qPCR assay da Silva et al. (2007)
Hepatitis A virus reverse transcription qPCR assay Costafreda et al. (2006)
Hepatitis E virus reverse transcription qPCR assay Jothikumar et al. (2006)
Murine norovirus reverse transcription qPCR assay Baert et al. (2008)
Treatment of water and effluent samples Wyn-Jones et al. (2011)
Incorporation of SPC (Diez-Valcarce, 2011a)
Treatment of soft fruit samples Dubois et al. (2006)
Treatment of salad vegetable samples Dubois et al. (2006)
Treatment of shellfish samples Henshilwood et al. (2003)
Treatment of liver tissue and pork meat samples Bouwknegt et al. (2007)
Treatment of faeces and animal-derived fertiliser samples Method devised by VITAL

Treatment of handlers' hands wash-off samples
Treatment of animal blood samples

The qualitative performance characteristics of the VITAL qPCR-based method to detect human adenoviruses in raspberries were determined through a collaborative trial involving eleven of the VITAL laboratories (D'Agostino et al, 2012). Sensitivity, or correct identification of 25 g raspberry samples artificially contaminated with between 5 × 102 and 5 × 104 pfu, was 98.5 %; the accordance and concordance were both 97.0 %. The positive predictive value was 94.2 %. The trial specificity, or percentage correct identification of un-artificially contaminated samples, was 69.7 %; the accordance was 80.0 % and the concordance was 61.7%. The negative predictive value was 100 %. The overall results of the collaborative trail were considered to show that the qPCR-based method for detection of human adenoviruses in soft fruits was acceptably robust to be used in the data-gathering stage of the project. Moreover, it was considered that the performance of this method would be representative of the performance of each of the detection methods that VITAL would deploy in the various food supply chains.

VITAL's sampling strategy

VITAL aimed to determine if there were any correlation between the presence of virus contamination and food safety practices in the various food supply chains. Each phase – Production, Processing, and Point of Sale - of the soft fruit, salad vegetable and pork supply chains would be sampled. Shellfish samples would be taken only at Point of Sale, as other international and national projects have studied virus contamination of shellfish at production and processing in depth, and much information was already available compared to the other food supply chains.

Data-gathering laboratories contacted at least one food business operator in each phase of each food supply chain, and gained their permission to take samples and analyse them for the presence of viruses. It was essential to ensure that the sampling points were realistic to the actual production situation. Phase-specific questionnaires were sent to the food business operators to complete; these questionnaires were key tools to identify the points within food premises (farms, slaughterhouses, retailers etc.) where the samples for virus analysis were to be taken. They were based on a WHO code of practice, EU legislation, national codes of practice, guidance notes and standards, industry best practice and scientific literature.

- World Health Organisation (WHO)
- Recommended International Code of Practice General Principles of Food Hygiene CAC/RCP 1-1969 (WHO)
- EU Legislation
- Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs
- Regulation (EC) No 853/2004 of the European Parliament and of the council of 29 April 2004 laying down specific hygiene rules for food of animal origin
- National codes of practice, guidance notes and standards
- Guide to Food Hygiene and other Regulations for the UK Meat Industry (FSA-UK*)
- Code of Practice for Food Safety in the Fresh Produce Supply Chain in Ireland(FSAI*)
- Code of Practice on the Risk Categorisation of Food Businesses to Determine Priority for Inspection (FSAI)
- EC Guidance Document on the Implementation of Procedures based on the HACCP Principles and on the Facilitation of the implementation of the HACCP Principles in certain Food Businesses (EC)
- Guidance Note on the Implementation of Food Safety Management Systems in Beef and Lamb Slaughter Plants based on HACCP Principles (FSAI)
- Guidance Note – Assessment of HACCP Compliance (FSAI)
- Guidance Note – EU Classification of Food (FSAI)
- Guidance note for Health Boards on the Inspection of a food Business (FSAI)
- National Guidelines on the Management of Outbreaks of Norovirus Infection in Healthcare Settings
- Irish Standard- Hygiene in the catering sector (NSAI*) Irish Standard – Hygiene in food retailing and wholesaling
- Safe Catering Package (FSAI)
- FSA-UK = United Kingdom Food Standards Agency; FSAI = United Kingdom Food Standards Agency; NSAI = National Standards Authority of Ireland.

Each questionnaire consisted 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. The

completed questionnaires were studied by the VITAL food safety management and risk assessment experts, who used the information contained in the questionnaires to identify the premises where sampling should take place, and the obvious points in those premises where samples should be taken. These points were termed "general" sampling points, and they were sampled during each sampling occasion.

In due course, fact-finding visits were made to the premises by the fact-finding team (see later). During the fact-finding visits, more points were identified where contamination with viruses could potentially occur. These were termed "ad hoc" sampling points, and samples were taken from them only during the fact-finding visit. At the moment of sampling, the actual standards of both operational and structural hygiene were documented and linked to the sampling results, and subsequently used as a basis for the development of the VITAL Guidance Sheets (see later).

Prevalence of viruses in food supply chains

Data-gathering lab WP2 Production WP3 Processing WP4 Point of Sale
CZ Pork, Soft fruit Pork, Soft fruit Pork, Soft fruit
ES Pork Pork Pork, shellfish
FI Soft fruit Soft fruit Soft fruit, shellfish
GR Salad vegetable Salad vegetable Salad vegetable, shellfish
IT Pork Pork Pork
PL Soft fruit, Salad vegetable Soft fruit, Salad vegetable Soft fruit, Salad vegetable
SR Soft fruit, Salad vegetable Soft fruit, Salad vegetable Soft fruit, Salad vegetable
UK Pork Pork Pork

The results of the analysis of the salad vegetable supply chain in the production phase are shown in Table 5. A total of 6 farms were sampled.

Table 5 Prevalence of viruses in the production phase of the salad vegetable supply chain

Sampling point HAdV PAdV BPyV HAV HEV NoV GGI NoV GGII
Irrigation water 17/61 6/39 2/39 0/35 1/20 0/35 1/25
Toilets/latrines 3/15 – – 1/9 – 2/9 1/8
Toilet doorhandles 4/13 – – 1/10 0/1 2/10 2/8
Harvesters hands 34/209 – – 2/97 – 0/94 1/101
Seasonal workers hands 1/30 – – 0/1 – 0/1 0/1
Manure 3/5 – – 0/2 – – 2/2

HAdV = human adenovirus; PAdV = porcine adenovirus; BPyV = bovine polyomavirus, HAV = hepatitis A virus; HEV = hepatitis E virus; NoV GGI = norovirus genogroup 1; NoV GGII = norovirus genogroup 2; – = not done.

The results of the analysis of the salad vegetable supply chain in the processing phase are shown in Table 6. A total of 3 processors were sampled.

Sampling point HAdV PAdV BPyV HAV NoV GGI
Handlers' hands 0/33 – – – –
Rinsing water 2/11 0/5 0/5 0/1 0/1
Knives 0/24 0/24 0/24 – –

HAdV = human adenovirus; PAdV = porcine adenovirus; BPyV = bovine polyomavirus, HAV = hepatitis A virus; NoV = norovirus; – = not done. No samples were analyzed for HEV and NoV GGII.

Sampling point	HAdV	PAdV	BPyV	HAV	HEV	NoV GGI	NoV GGII
Butterhead lettuce at farmers' market	4/120	1/110	0/120	0/120	4/119	2/120	1/120
Butterhead lettuce at supermarket	2/56	6/56	0/56	0/2	0/6	0/2	0/2
Romaine lettuce at supermarket	64/89	–	–	0/27	–	0/27	0/4

HAdV = human adenovirus; PAdV = porcine adenovirus; BPyV = bovine polyomavirus, HAV = hepatitis A virus; HEV = hepatitis E virus; NoV GGI = norovirus genogroup 1; NoV GGII = norovirus genogroup 2; – = not done.

Sample type	HAdV	PAdV	BPyV	HAV	HEV	NoV GGI	NoV GGII
Workers' hands	0/2	–	–	–	–	–	–
Equipment swabs	2/7	0/1	0/1	0/3	–	0/3	0/1
Water samples	0/9	2/9	1/9	0/2	0/5	0/2	0/2
Surface swabs	0/1	0/1	0/1	–	–	–	–
Toilet doorhandles	0/2	0/2	0/2	0/1	0/2	1/1	0/1
Toilet swabs	1/3	–	–	0/2	–	0/2	0/2
Produce	0/10	0/10	0/10	–	0/1	0/6	0/6

HAdV = human adenovirus; PAdV = porcine adenovirus; BPyV = bovine polyomavirus, HAV = hepatitis A virus; HEV = hepatitis E virus; NoV GGI = norovirus genogroup 1; NoV GGII = norovirus genogroup 2; – = not done.

Sampling point	HAdV	PAdV	BPyV	HAV	HEV	NoV GGI	NoV GGII
Irrigation water	9/95	4/89	1/89	0/56	0/56	0/56	2/56
Toilets/latrines	2/22	–	–	0/9	–	0/9	0/9
Toilet doorhandles	2/22	–	–	0/10	–	0/10	0/10
Harvesters' hands	10/72	–	–	0/15	–	0/15	0/15
Seasonal workers' hands	4/171	–	–	0/98	–	0/98	0/98
Pig faeces	–	4/7	–	–	0/4	–	–
Cattle Faeces	–	–	0/7	–	–	–	–

HAdV = human adenovirus; PAdV = porcine adenovirus; BPyV = bovine polyomavirus, HAV = hepatitis A virus; HEV = hepatitis E virus; NoV GGI = norovirus genogroup 1; NoV GGII = norovirus genogroup 2; – = not done.

A total of 4 processors were sampled.

Sampling point	HAdV	PAdV	BPyV	HAV	HEV	NoV GGI	NoV GGII
Handlers' hands	1/51	–	–	0/1	–	0/1	0/1
Conveyor belt	0/55	0/39	0/39	0/24	0/24	0/24	0/24

HAdV = human adenovirus; PAdV = porcine adenovirus; BPyV = bovine polyomavirus, HAV = hepatitis A virus; HEV = hepatitis E virus; NoV GGI = norovirus genogroup 1; NoV GGII = norovirus genogroup 2; – = not done.

A total of 7 premises were sampled.

Sample Type	Sample State	Sampling Point	HAdV	PAdV	BPyV	HAV	HEV	NoV GGI	NoV GGII
Raspberries	Fresh	Supermarket	0/77	4/10	0/10	–	0/4	–	–
		Farmers' market	1/60	0/60	0/60	0/60	0/60	0/60	0/60
	Frozen	Supermarket	2/58	1/40	0/40	0/2	0/1	0/2	0/2
		Processing unit	1/37	0/37	0/37	0/37	1/37	0/37	0/37

Strawberries	Fresh	Supermarket	0/4	0/4	0/4	0/1 –	0/1	0/1
		Farmers' market	1/47	0/47	0/47	0/20 –	0/20	0/20

HAdV = human adenovirus; PAdV = porcine adenovirus; BPyV = bovine polyomavirus, HAV = hepatitis A virus; HEV = hepatitis E virus; NoV GGI = norovirus genogroup 1; NoV GGII = norovirus genogroup 2; – = not done.

Sample type	HAdV	PAdV	BPyV	HAV	HEV	NoV GGI	NoV GGII
Workers' hands	1/7	0/2	0/2	0/4 –	0/4	0/4	
Hands under gloves	1/22 –	–	0/12 –	0/11	0/11		
Equipment swabs	0/12	0/1	0/1	0/6	0/3	0/14	0/14
Water samples	0/11	0/10	0/10	0/1	0/1	0/2	0/2
Surface swabs	0/4	0/3	0/3 –	–	0/1	0/1	
Toilet doorhandles	0/11	0/1	0/1	0/6 –	0/8	0/8	
Toilet swabs	0/12	0/1	0/1	0/7 –	0/10	0/10	
Faeces	0/1	0/2	0/2 –	–	–	–	
Produce	0/14	0/13	0/13	0/7	0/7	0/9	0/9

HAdV = human adenovirus; PAdV = porcine adenovirus; BPyV = bovine polyomavirus, HAV = hepatitis A virus; HEV = hepatitis E virus; NoV GGI = norovirus genogroup 1; NoV GGII = norovirus genogroup 2; – = not done.

Sampling point	PAdV	HEV
Pig faeces	144/153	35/153
Pig liver	6/152	6/152
Pig meat	1/153	3/153

PAdV = porcine adenovirus; HEV = hepatitis E virus

Sampling point	PAdV	HEV
Meat grinder	0/14	0/14
Sausages*	1/76	0/78

PAdV = porcine adenovirus; HEV = hepatitis E virus; * sausages were to be packaged and transported before being at display in retail stores

Sample Type	Sample State	Sampling Point	PAdV	HEV
Sausage	Raw	Supermarket	0/102	6/102
		Butcher shop	0/13	0/13
	Fermented	Production unit	2/93	6/93
	Fermented and smoked	Supermarket	1/92	0/92

PAdV = porcine adenovirus; HEV = hepatitis E virus

Sample type	HAdV	PAdV	HEV
Workers' hands	0/7	4/18	5/18
Hands under gloves	–	3/8	2/8
Equipment swabs	0/13	4/35	9/35
Effluent	–	2/6	0/6
Surface swabs	0/4	5/15	6/15
Toilet doorhandles	0/1	0/4	0/4
Toilet swabs	0/1	1/6	1/6
Liver	0/2	1/3	0/4
Black pudding	–	0/2	0/2

HAdV = human adenovirus; PAdV = porcine adenovirus; HEV = hepatitis E virus; – = not done.

Sample Type	HAdV	HAV	HEV	NoV GGI	NoV GGII
Mytilus galloprovincialis	34/51	0/102	3/51	0/102	23/102
Mytilus edulis	–	0/51	0/51	1/51	2/51

HAdV = human adenovirus; HAV = hepatitis A virus; HEV = hepatitis E virus; NoV GGI = norovirus genogroup 1; NoV GGII = norovirus genogroup 2; – = not done

Survival and elimination of viruses

Different geographical areas have significant differences in environmental conditions that may affect viral stability, the most significant factors being temperature, turbidity, and sunlight. The survival of human adenoviruses (HAdV) was studied in relation to temperature and solar irradiation in water matrices that may contaminate food and in lettuce and strawberry surfaces. The stability of viruses was analysed by infectivity assays in cell culture and by qPCR.

Mineral water microcosms and food surfaces were spiked and placed under artificial solar light and dark conditions at different temperatures for 24 hours. The results indicate that temperature is the main factor affecting HAdV stability in fresh produce surfaces. At 30oC, between 2 and 5 log10 of infectivity decay was detected both under dark conditions and sunlight exposure, whereas no decay was observed at 4°C under both conditions. Interestingly, in water microcosms a major role of sunlight affecting viral stability was shown. No viral inactivation was detected at 4, 20 and 37°C under dark conditions. On the contrary, between 2 and 4 log10 of infectivity decay was detected at the same temperatures after sunlight exposure.

The efficacy of intervention measures commonly used to inactivate pathogens in food industries was also studied. Chlorine disinfection was characterized by the efficiency factor Hom (EFH) model, a widely used model which takes account decreasing chlorine concentrations caused by the virus itself and organics present in the solution. The effect of chlorine in seawater, to be used in shellfish depuration, was analyzed by treating HAdV with an initial free chlorine concentration of 2.5 mg/L for up to 60 minutes. After 30 minutes when viral infectivity was analyzed, HAdV showed a 2 log10 reduction being still present after 60 minutes of chlorine disinfection. Similarly, buffered demand free water was prepared and spiked with human adenoviruses and treated by adding an initial free chlorine concentration of 2.5 mg/L for up to 60 minutes achieving a reduction of 4.5 log10. Other results of this study show that contamination of water by urban sewage may favour conditions where HAdV are much more resistant to free chlorine than expected.

Further studies characterized the effect of ultraviolet exposure on HAdV stability. At the end of the treatment the dose applied to the samples was 1400 J/m². The results show that HAdV are highly stable to UV-light irradiation, being inactivated by the treatment by approximately 2 logs. NoV persistence on fresh raspberries post harvest is challenged by the applied storage conditions such as temperature and relative humidity, the duration of storage (shelf life), and the fruit matrix itself. No intervention measures aiming to reduce the number of pathogens are applied in practice due to the perishability of the fruit. Therefore, survival of NoV GGII, NoV GGI, murine norovirus (MNV-1, a culturable surrogate of NoV), and HAdV, on raspberries, strawberries and in phosphate buffered saline (PBS) at temperatures (4oC, 10oC and 21oC)

commonly found in food supply chain settings was studied by molecular and cell culture techniques. Monophasic, biphasic and Weibull models were fitted to virus counts with maximum likelihood estimation. The tested viruses demonstrated the highest persistence in PBS followed by raspberries and then strawberries. D-values (the time required for the first 1 log10-unit reduction in virus titer) of all viruses exceeded or reached the shelf life of berries, however, at room temperature a sharp decrease in infectious MNV-1 and HAdV particles on strawberries was observed with D-values of only 1 day, and 2 days for NoV GGI based on the targeted genome fraction. Overall NoV GGII displayed more robust persistence than NoV GGI. The similar persistence of MNV-1 and human adenovirus, justifies, based on viral persistence, the use of HAdV as an indicator for the presence of NoV. The obtained results show moreover that already low contamination levels of the highly infectious NoV may be associated with an increased infection risk of humans after consumption of soft berries, especially raspberries, due to the great persistence of the virus in the food supply chain. The estimated decay rates and uncertainties of the study serve as important input requirements in the quantitative assessment of public health risks from consumption of soft fruits.

In addition NoV transfer from finger tips to raspberries, strawberries and lettuce and vice versa was studied. Besides being a vital perquisite for the QMRA in order to elucidate the role of food handlers in the transmission of NoV, the data also provides an idea about the distribution and concentration of viruses that can be expected on produce if food handlers are the source of contamination. This knowledge is in turn essential to evaluate the efficiency of possible and applied intervention measures.

During the course of VITAL, a novel cell culture propagation system was developed for hepatitis E virus, and used to study the survival of this agent under various conditions. HEV was shown to be able to survive heating at 56oC, exposure to UV light at levels used in butchers' shops, and a degree of resistance to sodium hypochlorite.

Effect of vaccination on the transmission dynamics of hepatitis E in pigs

HEV genotype 3 is endemic in commercial pig farms worldwide, and these pigs act as a reservoir. Pig-to-human transmission may occur when infectious animals enter the food chain at slaughter, through consumption of contaminated meat, direct exposure or use of by-products. To reduce the fraction of infectious animals at slaughter age and thus the risk for public health, it is important to understand the transmission dynamics of HEV in pig populations. VITAL estimated the transmission rate parameter and average infectious period of HEV in pigs from field data, using a combination of maximum likelihood estimation (MLE) and Monte Carlo sampling. The data were collected in ten commercial pig herds that are each divided into three different age groups.

Two transmission models were compared, assuming that animals are infected either locally by their group mates or globally by any infectious animal regardless of its group. For local and global transmission, the transmission rate parameters were 0.094 (median MLE, 0.074-0.12 credible interval of MLEs) day-1 and 0.11 (0.070-0.17) day-1, the average infectious periods were 40 (31-53) days and 43 (33-59) days and the reproduction numbers were 3.7 (3.0-4.8) and 4.7 (3.6-6.4). Based on these

results, global transmission is considered to be the more conservative model.

Three effects of vaccination were explored separately. When vaccination is not sufficient to eliminate the virus, a shorter average infectious period decreases the fraction of infectious animals at slaughter age, whereas a reduced transmission rate parameter adversely increases it. With a reduced susceptibility, vaccination of animals at a later age can be a better strategy than early vaccination. These effects should be taken into account in vaccine development.

Risk Assessment

The data on virus prevalence was analysed using modular process risk modelling. A modular process risk model consists of a series of interrelated parameters which are each described by a probability distribution. These distributions reflect the statistical parameter uncertainty given the observations made along the food chain. The eventual risk estimates are obtained by taking 50,000 Monte Carlo samplings from these uncertainty distributions of parameters and by subsequently calculating for each sampling the level of contamination (raspberries) or probability of an adverse health event (lettuce, pork sausage and mussels).

Table 18 shows the results for HAdV detection as observed in two raspberry production chains. Production chain A has the following identified potential contamination points at processing: water used for spray irrigation and harvester's hands (regular harvesters and seasonal workers). Production site B differs from site A with the use of drip irrigation and mechanical harvesting. The latter two practices were not considered to be potential contamination points for viruses and no other potential contamination points were identified. Raspberries from site A were not further processed except transportation on a conveyor belt to a location where they were frozen. At site B, raspberries were transported on a conveyor belt and potentially touched by food handlers before being frozen. As end-product frozen raspberries were sampled. The collected raspberries were processed along the same production chain.

Sampling point Site A Site B

Production

Irrigation water 0/19 -

Harvesters hands* 4/64 -

Processing

Conveyor belt 0/24 0/15

Food Handler - 1/51

Point of Sale

Frozen raspberries 1/37 2/28
regular harvesters and seasonal workers

The dotted and solid black lines in Figure 1a and 1b respectively represent the estimated contribution based on the chain model and the estimated virus contamination at point of sale. For both premises the two curves overlap, albeit slightly for site A specifically.

Vegetables: human infection risks with NoV due to consumption
Noroviruses were found in two lettuce head production chains. Data were sufficient to perform a quantitative risk assessment for one of those; sample sizes were too little for the other chain. The former production chain contained two identified potential contamination points: spray irrigation and contact between lettuce heads and harvesters' hands. Table 19 shows the estimated number of NoV infections (both NoV gg1 and gg2) for consumption of 150 g of lettuce based on the chain model and point of sale. No noroviruses were found in surface water and on harvesters' hands, giving a most likely estimate of zero NoV infections resulting from the consumption of lettuce heads produced at the time of sampling. However, noroviruses were found on lettuce heads collected at point of sale (3 of 120 lettuce heads), yielding risk estimates of 540 and 23 infections for unwashed and washed lettuce heads, respectively.

	Unwashed		Washed	
State at consumption	Mean	95% UL	Mean	95% UL
Chain model	0	57	0	2
Point of sale	540	~1,600	23	100

Pork: human infection risks for HEV due to pork sausage consumption
Pork was sampled in four production chains in four countries. Pig faeces liver and meat were collected from the same pig in three countries, allowing for the estimation of the conditional probability that pig meat is contaminated with HEV given a pig is infected. To this end, pigs were considered to be infected when at least one of the samples contained HEV PDUs. This approach mounted to a total of 31% of the tested animals being HEV-infected. From those infected pigs, three carried detectable HEV PDUs in meat. The conditional probability of HEV positive meat given a pig is infected is therefore 8.6% (95% confidence interval: 5.9% – 11.2%). The estimated HEV PDU concentrations for these three samples were 4×104 (95% CI: 9×103 – 1.4×105), 51 (95% CI: 3 – 227) and 7×102 (95% CI39 - 3×103).

The eventual risk posed by consumption of a pork sausage is, in addition to the HEV concentration, dependent on the amount of meat used per batch of sausages, the amount of meat used per carcass for sausage production, the prevalence of HEV infected pigs, other additives that dilute the HEV concentration and employed processes and conditions that lead to HEV inactivation. Table 20 shows the known and unknown information regarding the pork sausage production, as obtained from the producers in the respective countries. Because sausage composition is important for the magnitude of risks to consumers, and this information is unknown at present for countries C and D, the risk assessments for VITAL focuses on the sausages produced in countries A and B.

	Country A	Country B	Country C	Country D
Weight of pork per batch (kg)	75	11,400*	unknown	unknown
Number of pigs per batch	3 to 4	6,000	unknown	unknown
Sausage weight	140 / 300†	100	450	76
Sausages per batch	~1,700 / ~800	~120,000	unknown	unknown
Pork meat per sausage (g)	30 / 90	95	unknown	65

Pork liver per sausage (g) 42 / 90 0 0 0
Other ingredients pork fat (44%)
beef (12%) none specified salt; paprika; garlic; oregano; antioxidant; preservatives; cayenne pepper; possibly stuffed in pork intestine none specified
Processing Fermentation 8-24h 18-20 oC; Cold smoking for 12h consumed raw or cooked drying; curing cooked before consumption
- weekly production; † two types of sausages are produced

Shellfish: human infection risks due to mussel consumption
Shellfish were sampled in three different European countries. The estimated infection risks for the three countries are presented in Tables 21-24, both for the consumption of mussels as well as starters.

	Consumed raw		Consumed cooked	
State at consumption	Mean	95% UL	Mean	95% UL
Country A Chain model	12	28	4	10
Point of sale	0	1.4	0	0.5
Country B Chain model	23	70	9	26
Point of sale	0	11	0	4
Country C* Chain model	0	3.0	0	1.5
Point of sale	0.2	0.03 – 0.5	0.1	0.01 – 0.2
Country D* Chain model	0	3.2	0	0.9
Point of sale	0.03	0.005 – 0.1	0.01	0.002 – 0.03

- assumed to have the same sausage batch size and composition as country B due to lack of data for countries C and D

	State at consumption	Starter (19 mussels)		Dinner (60 mussels)	
Virus		Mean	95% UL	Mean	95% UL
HAV	raw	0*	0.04	0*	0.12
	cooked	0*	9×10-4	0*	3×10-4
HEV	raw	0.16	0.34	0.5	1.07
	cooked	0.06	0.12	0.18	0.38
NoV	raw	5000	7300	5000	7300
	cooked	490	901	1350	2500

- most likely

	State at consumption	Starter (19 mussels)		Dinner (60 mussels)	
Virus		Mean	95% UL	Mean	95% UL
HAV	raw	0*	5.9×10-4	0*	1.9×10-3
	cooked	0*	1.3×10-6	0*	4.0×10-6
HEV	raw	0*	2.8×10-4	0*	8.8×10-4
	cooked	0*	2.7×10-5	0*	2.4×10-4
NoV	raw	4900	7300	5000	7300
	cooked	57	112	176	330

- most likely

	State at consumption	Starter (19 mussels)		Dinner (60 mussels)	
Virus		Most likely	95% UL	Most likely	95% UL
HAV	raw	0	1.9×10-4	0	5.9×10-4

cooked 0 4.0×10-7 0 1.3×10-6

NoV raw 0 74 0 228
cooked 0 0.2 0 0.50

The estimated number of health events for HAV and HEV were low for all countries that examined these viruses. The estimated number of NoV infections was high in the countries where NoV was detected in oysters (countries A and B). The estimated average concentration per mussel for country A was approximately 16 (95% upper limit: 19.6) PDU per mussel; for country B this was about 10-fold lower, with on average approximately 2 (3.1) PDU per mussel. Due to the high infectivity of NoV that is implied by the dose-response model, the number of estimated health events for consumption of raw mussels is equal between countries A and B despite differences in the NoV concentration per mussel.

Comparison of risks

The results presented above show that the estimated probability of health events differs markedly between the production chains. No human pathogenic viruses were found in two soft fruit production chains and in one of the three lettuce head production chains. In contrast, lettuce heads produced in the other production chains were contaminated with NoV and infections were estimated to occur with the risk assessment models. Furthermore, differences between estimated health risks for NoV compared to HAV and HEV were observed in mussels obtained in supermarkets, and for HEV in pork sausages.

The value of such comparisons between estimated health risks, however, is limited for prioritizing the viruses at present. An important argument for this statement is the difference between the virus-specific dose response models. The dose-response model for NoV is based on the highly infectious variant of NoV, Norwalk virus, and the response measured was infection after ingestion of the inoculum by human volunteers. The dose response model for HAV is based on the inoculation of institutionalized children with a faecal HAV-suspension and monitoring for the development of jaundice. The dose response model for HEV was based on intravenous inoculation of pigs with HEV, corrected to reflect infection following the faecal-oral exposure route. To enable a more appropriate comparison between different viruses, these aspects need to be considered quantitatively.

In conclusion, VITAL has shown that viral contamination in each of the different food production chains occurs. In addition to large epidemiological studies that have been conducted and show a significant number of people ill associated with the consumption of virus contaminated foods, the use of MPRM based risk assessments has added value. It was shown here that the estimated health risks were negligible in some cases (e.g., HEV in shellfish) and significant in others (e.g., NoV in shellfish or HEV in pork sausage) when consumption and dose-response were considered in combination with the data on virus concentrations in different sources and foods along the food production chains. Based on the detection of viruses, HAV was less frequently found in the food chains than HEV or NoV. Of the latter two, NoV was most frequently detected. These findings may however be biased by the sampling scheme as described before. Similarly, differences in the dose-response models used for risk quantification hamper a prioritization of the most important viruses in the examined food production chains. Nevertheless,

viruses were found in the food production chains of which food products are consumed raw by European consumers. The relevance of studies to reduce viral contamination in these food production chains is therefore shown to be eminent.

Viral Food Safety Management

The final and overarching aim of VITAL was to facilitate the development of new measures to prevent virus contamination of foods, and for virus reduction and control in case of virus contamination. The ultimate analysis of all the findings of the project should bring this aim closer to fulfilment.

The results of sample analysis obtained by the data gathering laboratories and the areas of concern (AOCs) , i.e. non-compliance with good practices, e.g. Good Agricultural Practice (GAP), Good Hygiene Practice (GHP) etc. identified by the fact finding team were integrated by cluster analysis of the different sampling sites and correlation analysis of the identified clusters and positive samples. This was done in order to identify links between positive samples and AOCs, with the aim of determining which non-compliances with prerequisite programs such as GAP or GHP could open vulnerabilities in the food supply chains to virus contamination.

At time of preparation of this report, much of the extensive amount of information obtained from the sample analysis and the fact-finding missions is still being analysed. Initial results of the analysis of the AOCs in the soft fruit and salad vegetable supply chains has revealed that relatively more areas of concern were identified in production companies as compared with both processing companies and companies at point-of-sale for all of these categories. In addition, among these areas of concern, there was a higher ratio of significant areas of concern over minor areas of concern for production companies. Notably, in primary production of soft fruit and salad vegetables the analysis of AOC clusters and virus contamination data has revealed correlation between key non-compliances (use of poor quality irrigation water, poor sanitation, poor hand hygiene) and contamination of produce.

During the course of the VITAL project, the 40th Session of the Codex Committee on Food Hygiene in December 2008 set in motion the first international work on a Code of Hygienic Practice for the control of viruses in food, entitled "Proposed Draft Guidelines on the Application of General Principles of Food Hygiene to the Control of Viruses in Food". These guidelines follow the format of the Codex Recommended International Code of Practice - General Principles of Food Hygiene- (CAC/RCP 1-1969), and contain the sections of that document which are relevant to the soft fruit, salad vegetable, and shellfish supply chains. VITAL compared the AOCs identified during the fact-finding visits with the recommendations of the proposed Codex virus guidelines. The proposed guidelines cover all areas of concern identified by VITAL. VITAL has determined that in particular compliance with prerequisite programs is essential to reduce the risk of contamination of food supply chains with viruses, and as such the Codex Guidelines do not need major amendments in structure. However, correlations between AOCs and the results of sampling have identified some AOCs, e.g. hand hygiene, and water quality, which are of critical importance for control of virus contamination and therefore should be strongly emphasized by the Codex guidelines.

To complement the Codex guidelines, and assist in compliance with prerequisite safety programs, three Guidance Sheets were developed, as aids to prevent virus contamination of the salad vegetable, soft fruit, and pork product supply chains. The Guidance Sheets contain recommendations based on accepted good practices and augmented by findings from the analysis of critical points performed during the VITAL fact-finding missions. The Guidance Sheets are intended for dissemination to the food industry, and have been placed on the public pages of the project website.

In primary production of berry fruits and leafy greens, the correlation between key non-compliances (poor quality irrigation water, poor sanitation, poor hand hygiene) and contamination of produce can allow significant recommendations to be made (the ongoing analysis of the VITAL findings will allow this to be done in due course for the pork supply chain). The potential key virus-significant Critical Control Points are irrigation water and workers' hands. The system is out of control when irrigation water is not fit for its intended purpose or poor hand hygiene is evident. Control measures should be: fuller compliance with prerequisite programs. Monitoring water and hands for viruses will verify the condition of the system, and therefore should be integrated into existing food safety systems. The results of VITAL may indicate that virus-specific process hygiene criteria could be considered, for example zero enteric viruses per 10 liters of irrigation water, or zero enteric viruses in a sample of handler's hands. Adoption of such criteria, with the clear recommendations on regaining control through compliance with prerequisite programs, would fulfill the aim of integrated monitoring and control of foodborne viruses in the food supply chains.

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Potential Impact:
This project aims to assist in the development of national policy, strategies and action plans aimed at improving food safety and trade through the application of good practices as it concerns the virological contamination of fresh food throughout different European food chains. It identifies the vulnerabilities to viral contamination in each food supply chain and provides guidelines based on the experience of experts as well as data analysis in their respective countries. In this regard, new flexible approaches which frame to the implementation of HACCP systems for viruses are described. The final and overarching aim of VITAL was to facilitate the development of measures to prevent virus contamination of foods, and for virus reduction and control in case of virus contamination. The ultimate analysis of all the findings of the project should bring this aim closer to fulfillment.

Approaches that could be adopted by European countries to improve food safety by facilitating guidelines for virus prevention were elaborated within this project. The new data about viruses is applicable to food supply chains engaged in food processing and preparation, distribution and storage, wholesale, and retail. With regard to primary food production (animal husbandry and on-farm activities), they could also be of important assistance to countries applying HACCP-based systems at farm level.

Appropriate guidelines are presented for use within the development of policy, strategy and action plans. The aim is not to directly provide absolute solutions to all issues (public health, economic etc.) regarding foodborne viruses but to help prevent virus contamination in the food supply. Adaptations of the Codex HACCP system used by national governments for virus contamination should be further considered by interested parties. It is stressed that the guidelines provided (based on the acquired data) need to be adopted and tailored, taking into account national circumstances: no single solution is the optimum choice in all situations.

The guidance documents are for use by governments, national bodies, food premises, companies developing national policy aimed at the application of specific preventive measures, and by professionals advising on national policy development (e.g. government officials, food industry associations, consultants, auditors, trainers/education specialists). However, they may also be of use to other groups of people, for example, food business managers and food enforcement officers. Together with GHP, HACCP, the guidelines should be recognized as an appropriate and useful tool for enhancing the virus safety of food products and providing increased food safety assurance.The guidelines which are based on risk assessment conclusions of the project are for use by all food businesses. There could be a greater uptake of these guidelines (and hence improved controls) in larger food businesses often involved in the export market. Food businesses play a vital role in adopting food safety management systems and are a key stakeholder in food safety policy development. In addition to the action taken by the food businesses themselves, governments are responsible for creating a scientific, technical and financial environment favorable to specific viral guidelines implementation.

In general, it is recognized worldwide that the HACCP system, although not specific for viral hazards, provides clear benefits to food businesses. It enhances the safety of food, and reduces the incidence of

foodborne disease. Additional benefits resulting from the implementation of the VITAL guidelines should be that staff and business owners gain confidence and are better equipped for informed discussion on virus food safety measures with food inspectors, third party auditors, consultants, trading partners, consumers and others. A virus-preventive system is essentially a management tool and its development requires a long term investment. This should result in more efficient use of staff and provision of adequate documentation. The increased level of process control can result in product consistency and improvements in traceability, with beneficial cost implications for companies as access to some markets is increased and more customers are attracted. The adoption of virus-relevant guidelines could be a valuable team-building exercise for a company or food industry, leading to improved education and awareness of staff working in farms/industries, in turn having a positive effect on the development and improvement of the enterprise as it demonstrates an ability to manage consistent changes in primary production as well as along food supply chains.

The effective application of the guidelines will depend on all the basic prerequisite programmes (i.e. HACCP, GHPs) being in place in a food business. The basic GHP programme is of prime importance for food safety, as stressed in the fourth revision of the Annex on HACCP (contained in the Codex General Principle of Food Hygiene – FAO and WHO, 2003). While following these guidelines and considering the national policy options for the application of HACCP or virus guidelines in the small business sector, it is necessary to take account of the existing food hygiene controls in the food business sector being targeted.

The VITAL project achieved its findings through an integrated assessment of food production practices and viral analyses. The project delivered some new insights on viral contamination routes which are important for the general public, and also delivered useful protocols for virus management via good hygienic practices in plant and animal production. The VITAL guidelines will in future be augmented by identification of critical control points and CCPs and control charts, to maintain the impact of the project on European food safety.

List of Websites:

http://www.eurovital.org