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Summary Report of Joint Scientific Workshop on Foodborne Viruses

Centre for Environment, Fisheries & Aquaculture Science

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Abstract

This report describes the outcome of a workshop held jointly by the Food Standards Agency UK and the European Food Safety Authority on foodborne viruses. The workshop gathered together academics, clinicians, veterinarians, food industry specialists and regulators with established expertise in epidemiology, detection and control of norovirus, hepatitis A virus and hepatitis E virus in foodstuffs. The primary objective of the workshop was to identify priority areas for future research funding in order to maximise efficiency and to benefit from synergies provided by interdisciplinary collaborations. This report describes the methodology employed to rank and prioritise research needs and the main workshop conclusions. The conclusions identified that the highest priorities were development and validation of methods for assessing hepatitis E virus infectivity, establishment of the relationship between the detection of norovirus in food and public health risk, development of methods for hepatitis E virus detection in meat and meat products, and determination of the burden of hepatitis E in human populations in Europe.

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Key words: Norovirus, Hepatitis A, Hepatitis E, Food **Correspondence:** michelle.price-hayward@cefas.co.uk



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Summary

A joint workshop on viruses in foods was held by the Food Standards Agency (FSA) and the European Food Safety Authority (EFSA) in February 2016 in order to bring together experts from research environments, clinical settings and food producing/processing operations to discuss the current state of understanding with regard to the three foodborne viruses currently of greatest public health concern: norovirus, hepatitis A virus and hepatitis E virus.

The workshop broadly followed the EFSA colloquium format, with opening addresses from FSA Chief Scientific Adviser Prof. Guy Poppy and EFSA Head of the Biological Hazards and Contaminants Unit Dr. Marta Hugas. Plenary talks from invited speakers followed, providing an introduction to each of the breakout session themes. These were followed by work in breakout sessions and then a final plenary session to bring all the group conclusions together.

The format of the workshop followed an adaptation of EFSA's Expert Knowledge Elicitation process; input was gathered from all participants followed by structured discussions with a focus on reaching consensus on the ranking of the research priorities. Each breakout session was led by an EFSA facilitator trained in this elicitation method and supported by rapporteurs from EFSA, FSA and Cefas. The views of all participants were registered via a system of voting cards, which were tabulated and statistically analysed by the facilitators in order to highlight where there was agreement and disagreement amongst the experts and to identify the areas of consensus.

Breakout sessions were held on the following themes:

- 1. Norovirus epidemiology;
- 2. Hepatitis A virus epidemiology;
- 3. Hepatitis E virus epidemiology;
- 4. Norovirus and hepatitis A virus methodologies;
- 5. Hepatitis E virus methodology;
- 6. Norovirus and hepatitis A virus control options;
- 7. Hepatitis E virus control options.

Each breakout session examined previously suggested research priorities and determined whether these were extant or whether there were now new priorities. Participants were asked to evaluate the resulting list of research ideas on the basis of their impact on public health in Europe and the feasibility of their implementation. They then ranked these in order of priority via the means described above. In the final plenary session, the participants then voted for overall research priorities from the top ranked ideas from each breakout session.

The key conclusions of the workshop were: (i) in the area of foodborne viruses there is a need to move beyond presence/absence methods to quantification and infectivity assays in order to better understand the potential risks to the consumer and the burden of disease this represents so that appropriate controls in the food chain can be applied. (ii) fundamental research is required to understand the role of food production in the transmission of hepatitis E virus in regions with modern water sanitation.

The following main priorities for improving the state of knowledge regarding norovirus, hepatitis A virus and hepatitis E virus in foods were identified following the elicitation process:

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- 1. The development and validation of direct and indirect methods for assessment of hepatitis E virus infectivity;
- 2. Establishing how the detection of norovirus in foodstuffs relates to public health risks;
- 3. Development of methods to evaluate norovirus and hepatitis A infectivity from food samples;
- 4. Development of standard methods and ISO methods for detection of hepatitis E virus in meat and meat products;
- 5. Establishing the burden of hepatitis E virus infections in humans in Europe.

The workshop closed with contributions from FSA and the European Commission Directorate General for Research and Innovation (DG-RTD) on potential means for funding projects in the scope of the identified priorities. Potential for collaborative working across research and industry was highlighted, and it is anticipated that by working together progress can be made against the high priority needs identified.

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1. Introduction

The joint UK Food Standards Agency (FSA) and European Food Safety Authority (EFSA) workshop on foodborne viruses was held in London, UK on 23-25 February 2016 and addressed the themes of epidemiology, detection methodology, and control options across the three main viral causes of foodborne illness: norovirus (NoV), hepatitis A virus (HAV), and hepatitis E virus (HEV).

Co-funding was provided through a grant from EFSA and contract funding from FSA to the Centre for Environment, Fisheries, & Aquaculture Science for the organisation and reporting of this workshop.

The workshop themes were developed by a scientific organising committee comprised of the following international experts and representatives from the funding agencies:

Sérgio Potier Rodeia (EFSA), Alisdair Wotherspoon (FSA), Giorgia Albieri (FSA), Pirkko Tuominen (Finnish Food Safety Authority), Reimar Johne (German Federal Institute for Risk Assessment [BfR]), Anne Thebault (French Agency for Food, Environmental and Occupational Health & Safety [ANSES]), Albert Bosch (University of Barcelona), Ines Skoko (Croatian Veterinary Institute), Olaf Stenvers (Netherlands Food and Consumer Product Safety Authority [NVWA]).

1.1. Background and Terms of Reference as provided by the requestor

Illness associated with microbial contamination of food has been known for decades and risks from bacterial contamination of foods have been, in many cases, well characterised and risk-managed. However, it has been recognised that the particular risks posed by viruses, which may behave very differently than bacteria, are rather more poorly characterised and controlled. In many cases, the control methods applied for bacterial contamination of foods are inadequate for the control of viruses (Richards, 1985). In 2007, the World Health Organisation Food and Agriculture Office (WHO/FAO) convened an expert meeting on the state of knowledge in the area of foodborne viruses in order to inform risk managers of the virus/food combinations that posed a particular risk and the options available to them for control of those risks (WHO/FAO, 2008). HAV and NoV in bivalve shellfish, fresh produce, and prepared foods were identified as the highest risk pathogens and commodities.

In 2011, EFSA published a scientific opinion on the current state of knowledge with regard to foodborne viruses, which provided an extensive review on the subject. This was followed in 2015 by a review of viruses in the food chain undertaken by the FSA Advisory Committee on the Microbiological Safety of Food. Results of this review were presented by the UK at the 55th meeting of the Advisory Forum on 04-05 March 2015. The report identified the three most important viruses associated with foodborne infection as NoV, HAV and HEV, and posed questions on areas of possible cooperation. EFSA presented an overview of activities of the Biological Hazards and Contaminants Unit on the subject. Poland, Latvia, Croatia, France, Germany, Spain, Sweden, Belgium, Finland and Slovakia also shared information on the subject, noting the need for a 'one health' approach involving the different disciplines of veterinary and public health expertise. Member States expressed interest in working collaboratively on issues that would support risk assessment in this area.

The issue was further discussed between EFSA and the FSA at the end of March 2015 in the context of a visit by EFSA's Executive Director to FSA. It was then agreed to further explore the setting up of a joint project on foodborne viruses, with a focus on NoV, HAV and HEV. EFSA expressed interest in providing financial support for a workshop on the subject, to be hosted in the UK, and with the participation of other interested Member States. The UK was chosen to host the workshop due largely to the work being carried out at the European Union Reference Laboratory (EURL) for monitoring bacteriological and viral contamination of bivalve molluscs, the Cefas Weymouth Laboratory, which, given its scientific excellence, technical knowledge and structural capacity, was the organisation of choice for undertaking the local arrangements.

This contract/grant was awarded by EFSA to: The Centre for Environment, Fisheries & Aquaculture Science (Cefas).

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Contractor/Beneficiary: The Centre for Environment, Fisheries & Aquaculture Science (Cefas).

Contract/Grant title: EFSA/FSA international workshop on viruses in foods.

Contract/Grant number: GA/EFSA/AFSCO/2015/03 Cefas.

1.2. Interpretation of the Terms of Reference

FSA contacted Cefas in August 2015 regarding a proposal to host a workshop together with EFSA on foodborne viruses. FSA were keen maximise the impact, relevance and practical outcomes for the public benefit of any applied research and development investment in this area. EFSA participated in the workshop in light of the relevant input it was able to provide following previous and on-going work and the high interest it had in the outcome.

The purpose of the workshop was to bring together experts in foodborne viruses in order to develop a ranked list of research priorities considering epidemiology, methods and control options for the viruses most often implicated in foodborne transmission of illness. EFSA offered a grant for the organisation of a workshop on foodborne viruses with a focus on NoV, HAV and HEV, and organised along three main streams: epidemiology, methodology and control options.

The scientific organising committee was established through agreement between FSA and EFSA. This committee comprised experts in the field of foodborne virology as well as representatives of FSA and EFSA. The committed then agreed the themes and organisms to be addressed in the workshop, as well as the duration of two half-days plus one full day, and the desire that it would be held in a UK location that would be easily accessible to experts travelling in from around Europe and other parts of the world. Cefas were identified as the desired organisers due to their expertise in the area of NoV methodology and food safety of bivalve molluscan shellfish.

Attendance at the workshop was ensured through direct invitations based on recommendations from members of the organising committee. Invitees comprised mainly scientific and technical experts from academia and governmental research institutes. FSA believed that it was also important for the control options to extend beyond regulatory solutions, and therefore appropriate expert input from the food processing industry was sought.

The workshop was planned to take place on the 23-25 of February 2016 in London.

1.3. Additional information

The invited speakers provided the following abstracts for their talks on the first plenary session of the workshop.

Plenary 1: Norovirus epidemiology and public health impact

Marion Koopmans, Erasmus Medical Centre, NL

The recently published global burden of disease estimate ranked noroviruses among the top causes of foodborne disease. Noroviruses are a highly diverse group of viruses, belonging to the family *Caliciviridae*. Although disease in humans has been associated with viruses belonging to three of the genogroups (GI, II and IV), and up to 40 genotypes, the vast majority of disease episodes is caused with a limited number of NoV genotypes. A challenge in estimating the proportion of NoV attributable to food contamination is the rapid person to person spread seen for some of the NoV genotypes, particularly GII3 and GII4. These viruses evolve upon circulation in the community through selection of variants that escape prior immunity. Greater diversity is seen when studying foodborne disease outbreaks, thought to reflect in part global virus diversity, selective advantages of GI noroviruses over others in terms of environmental survival, and possibly mixing with viruses from non-human sources.

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In addition to genetic drift, noroviruses evolve through genetic recombination, and their epidemiology is shaped though such evolutionary events.

The changing demography and increasing size of immune-compromised populations in healthcare settings recently has brought a new disease phenotype to attention, with chronic intermittent diarrheal disease and persistent NoV shedding in such patients. Against this backdrop, the great stability of noroviruses, the globalization of the food market, and the increasing difficulty in producing fresh foods free from environmental contamination, constitutes a significant public health risk. In addition, the spread of noroviruses in healthcare settings and through the food chain serve as important models for the emerging infectious diseases, as many of these are of zoonotic origin and have been introduced into the human population through food production.

Plenary 2: Hepatitis A epidemiology and public health impact

Rosa M. Pintó, University of Barcelona, ES

HAV is a somewhat neglected disease but is still the most common type of acute hepatitis worldwide. Although HAV is an infection of developing countries, there is a re-emergence of the disease in many developed countries due to the lack of herd immunity. Outbreaks associated to food imports from endemic countries contribute to this re-emergence. HAV genotypes show a geographical distribution, IA and IB being the most common worldwide. Genotype IIIA is also very frequent in the South Asian continent from where it is rapidly spreading. This spreading is of public health concern since genotype IIIA seems to induce a more severe disease.

HAV exists as a single serotype but improper vaccination schedules in immunosuppressed patients may prompt the isolation of antigenic variants with an important public health impact. Additionally, it should be noticed that there is an increasing concern regarding the emergence of a new serotype through zoonotic transmission.

Plenary 3: Hepatitis E epidemiology and public health impact

Harry Dalton, Royal Cornwall Hospital and University of Exeter, UK

Until recently, HEV was thought not to occur in developed countries. It is now clear that locally acquired HEV is common in many developed countries. HEV infection acquired in these areas differs from that in developing countries in a number of important aspects: it is caused by genotype 3 (and 4 in China and Japan); it mainly affects middle-aged/elderly males; it is zoonotic with a porcine primary host. Pig herds worldwide are infected with HEV genotype 3 and HEV has been found in the human food chain in a number of developed countries. However, the route of transmission is not fully understood, since most cases are not obviously associated with pigs/pig products. HEV can be transmitted by blood transfusion and surprisingly high numbers of asymptomatic blood donors are viraemic at the time of donation: Germany 1:1200, Netherlands 1: 600, England 1:2848.

Our understanding of the clinical phenotype of HEV infection in humans has undergone a sea-change in recent years. Previously, HEV was thought to cause only acute self-limiting hepatitis. However, HEV may cause persistent disease in the immunocompromised. Patients with chronic HEV infection have no symptoms, but some develop rapidly progressive liver cirrhosis. The full clinical spectrum of HEV is still emerging. HEV has important extra-hepatic manifestations, which deserve further investigation. For example, HEV can cause a wide range of neurological illness. In particular, very recent data suggests that Guillain-Barré syndrome and neuralgic amyotrophy are associated with locally acquired HEV in approximately 5% and 10% of cases respectively.

The incidence of HEV infection is much higher than previously thought. For example, there are thought to be >100,000 infections in England each year, but in 2015 only 800 cases were laboratory confirmed. This means that most infections with HEV3 are asymptomatic or unrecognised. In Europe

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there are documented 'hot-spots' for HEV, including SW France, the Netherlands, Czech Republic, and possibly central Italy. However, the burden of disease across Europe is uncertain due to the emerging nature of the clinical phenotype, and incomplete epidemiological data.

Plenary 4: Methodology for detection of Norovirus and Hepatitis A virus in foods; current status and future challenges

James Lowther, Centre for Environment, Fisheries & Aquaculture Science, UK

NoV and HAV are amongst the principal agents of foodborne illness and the introduction of testing for these viruses into food hygiene legislation or procedures for investigating foodborne outbreaks is currently a priority for many international, national and regional authorities. Methods for the detection of these viruses from food using the reverse transcription - polymerase chain reaction (RT-PCR) for amplification of viral RNA have been available for more than two decades however until recently there has been a lack of availability of standardised methods. In 2006, 23 international laboratories participated in a ring trial using methods for extraction and detection of NoV, which highlighted the need for development of a standardised method. In 2013 the International Standards Organisation (ISO) and European Committee for Standardization (CEN) published a joint technical specification for detection and quantification of viruses including NoV and HAV in foods using real-time RT-PCR (ISO/TS 15216). This method has subsequently been subject to an international validation and is due to be republished as a full standard in 2016.

The standard method accommodates the testing of various food matrices through the use of separate pre-processing and virus extraction methods for each food matrix, and operates parallel protocols for qualitative detection and quantification. Validation was undertaken in seven food matrices, including soft fruit, salad vegetables, bivalve shellfish and bottled water.

Future challenges in this area include progress on further international harmonization of methodology, improvements in the reproducibility of testing, and the development of methods for determination or estimation of virus viability. These latter may include improved virus culture methods, methods for determining genomic and/or capsid integrity, and the use of viable indicator viruses.

Plenary 5: Detection methods for Hepatitis E virus in food

Reimar Johne, National Reference Laboratory BfR, DE

Foodborne infection is increasingly recognized as an important route of transmission for HEV. However, no standardized methods are available for detection of HEV in food so far. Especially for porcine meat, liver and products thereof, which are known to be potent HEV transmission vehicles, harmonized detection methods are urgently needed.

For detection of the HEV genome in meat and meat products, several protocols applying different methods for sample preparation and nucleic acid extraction followed by (real-time) RT-PCR have been published. They show varying sensitivities depending on the applied method and the analyzed food type. Using those methods, HEV genome detection rates between 4% and 22% were determined for pork liver sausages from retail in Spain, UK, France and Germany. Inter-laboratory validation studies for some of the methods are ongoing.

Several efforts have been made to detect infectious HEV. Experimental inoculation of food preparations into pigs followed by measurement of HEV excretion and seroconversion was used in a few studies. However, high costs and ethical concerns argue against a broad use of this system. Isolation of HEV in cell culture is still difficult, rarely reproducible and mostly inefficient. Isolation of infectious HEV from pork liver sausage using a 3D cell culture has been described; however, the system is sophisticated and has a varying efficiency. Recently, cell culture systems using novel cell

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types have been published. In addition, the isolation of more efficiently replicating HEV strains from chronically infected patients has been described, which may be used for HEV stability testing in future.

Plenary 6: Norovirus and Hepatitis A - Control options for viruses in the food chain

Lee-Ann Jaykus, North Carolina State University, USA

There are two primary ways to control enteric virus contamination in the food chain: prevention and inactivation. The general principle for prevention is separation of human waste from food production and preparation. While theoretically simple, this is complicated by the complexities of controlling human behaviors and the absence of reliable indicator organisms that can be used in risk management. From the inactivation standpoint, HAV and NoV are environmentally persistent and have a high degree of resistance to commonly used virucidal compounds. When present on or in foods, they are able to withstand most conventional processing and preservation methods. A significant amount of work is being done to evaluate novel ways to prevent contamination and inactivate enteric viruses on surfaces, hands, and foods.

There is a clear need to prioritise foods and viruses based on their public health impacts. Imported foods pose a difficult problem, particularly where food supply chains are complex. Many of the potential control methods pose significant challenges with regard to commercial viability, practicality and impact on product quality. Prevention should always be the first priority, followed by inactivation.

Development of a mammalian cell culture model would be a key development in further study and determination of risk to public health. There is also a need for development of better molecular detection methods and of risk models for prioritization and scenario analysis. With regard to controlling contamination in the food production and processing environment, the role of food handlers needs to be addressed and in particular behaviours that lead to contamination.

With regard to reducing the risks from higher risk food products, such as shellfish and fresh produce there is a clear need to understand the sources and indicators of contamination and to develop effective, inexpensive, and practical virus inactivation methods that are pre-tested relative to sensory quality and shelf-life of the product.

Plenary 7: Hepatitis E virus – Transmission and control in the food chain

Wim van der Poel, Central Veterinary Institute Wageningen University, NL

HEV genotypes 3 and 4 have zoonotic potential and cause single cases of hepatitis throughout the world. Both of these genotypes have a main reservoir in domestic swine and this leads to contamination in the food chain. The virus may be transmitted to humans by different types of foods including meat products, and as environmental routes may be involved, also water, shellfish fruits and vegetables. Reducing HEV in the animal reservoir will not be an easy task and, to tackle this, a vaccination program will be needed. However, in Europe there is no vaccine available yet.

Therefore, HEV control options will have to be focused on the food chain. HEV is a relatively stable non-enveloped virus, and may remain infectious at 71°C, chlorine treatment and UV light. Focal points for control along the food chain depend on the type of food and the stage in the food production process. For better food safety and public health protection studies on the control of HEV need to be increased.

2. Data and Methodologies

Expert Knowledge Elicitation (EKE) provides a structured approach to the collation of opinions from expert groups in a transparent manner, which can be fully documented. EFSA developed guidance on

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the methodology focusing primarily on probabilistic approaches for eliciting expert judgment on quantitative parameters whilst minimizing bias (EFSA, 2014). These methods are aimed at obtaining values to support quantitative risk assessments through careful planning of elicitation exercises from initiation to post-elicitation reporting and statistical analysis. This workshop did not seek to obtain quantitative values for risk assessment models, but the core principles (planning, minimizing bias, documentation and transparency) of the method can also be applied to prioritisation exercises with some adaptations. Since the elicitation was to be performed at a face-to-face meeting, EFSA applied an adaptation of the Sheffield Elicitation Framework (SHELF) (http://tonyohagan.co.uk/shelf/), which uses behavioural aggregation to elicit the knowledge from a group of experts meeting face-to-face and distribution representing the uncertainty in the common judgements.

A list of previously identified priorities in foodborne virus research was developed on the basis of comprehensive reviews undertaken by both the FSA and EFSA on the occurrence and control of foodborne viruses (FSA, 2015; EFSA, 2011). This list of identified priorities was circulated amongst the organising committee for comment and agreement and was provided to the invited experts prior to the workshop.

To ensure a consistent approach between breakout groups when prioritising research ideas, all groups used the same evaluation criteria by its facilitator:

- 1. Impact on public health in Europe;
- 2. Feasibility of implementation;

A third evaluation criterion, given below, was held in reserve and only used if a group identified it as an important factor.

3. Innovation of the research.

Each breakout session began with clarification of the evaluation criteria and the identified list of priorities to ensure all participants had a common interpretation of the criteria and research ideas. Groups could then identify whether different or additional research priorities needed to be discussed. If the participants knew of on-going projects that covered a particular research priority the group discussed whether this research idea should still be included. In some groups, this led to substitution of some or all of the priority areas from the list. Some groups chose to combine the priorities into fewer, broader areas of research. Each participant then ranked the research priority list separately for each of the criteria.

Individual scores were tallied and box plots of the individual rank scores per research area were presented sorted by the median score to illustrate the degree of consensus for each of the research priorities according to each evaluation criterion (the results are presented in Annex B). The results were presented to the group for further discussion and members whose rankings differed significantly from the group consensus were asked to share their rationale. This stimulated further discussion about the overall rank for each research priority. There was an option for groups to undertake a further round of scoring, which allowed group members to revise their rankings if they so wished. This was not mandatory and only a few participants chose to rescore. The research ideas were ranked by the mean score for each criteria and an overall ranking calculated. This is presented in tabular form in the description of results by breakout session in Section 3 and in Annex C. Items with a score of one were considered to be the highest priority. Work in the breakout groups was followed by a brief presentation in plenary of each breakout group's conclusions.

The priorities, as ranked by the breakout groups, were then placed on flip chart easels around the room and each participant was given three stickers, colour-coded by the breakout sessions in which they sat, to place on their choice of the three 'top' priorities amongst the other six breakout session themes (*i.e.* they could not vote for priorities arising from their own breakout sessions). These scores were recorded and then presented in plenary on the following morning.

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On day three, participating experts were encouraged to sit at mixed tables so that they were with others from the different breakout sessions. Results of the previous evening's voting exercise were presented and followed by a facilitated plenary discussion of the outcomes. Each table was provided with a scoring sheet on which they cast allocated points to their top five research priorities by virus using the following scoring system:

- a) Five (5) points to the first priority;
- b) Four (4) points to the second priority;
- c) Three (3) points to the third priority;
- d) Two (2) points to the fourth priority;
- e) One (1) point to the fifth priority.

Scores were recorded and tabulated by the lead facilitator, resulting in an overall ranking of research priorities across the seven themes. Overall results were then revealed before closing the workshop with presentations.

Bar charts reflecting the level of consensus on the relative importance of the agreed list of research priorities for each breakout session are presented in Annex B. These bar charts also show the spread of opinion giving an indication of the intra-expert variation, and therefore, of the uncertainty in the expert judgement for each assessed ranking.

3. Assessment/Results

The results and summaries of the discussions and outcomes from each breakout session are presented below.

3.1. Breakout Session 1 – Epidemiology and public health impact of norovirus

Facilitator: Jose Cortinas Abrahantes Rapporteur: Rachel Hartnell/Kirsten Stone

Noroviruses are single-stranded (ss) RNA viruses of the family *Caliciviridae*. Noroviruses are most commonly classified according to the nucleotide sequence that codes for a capsid structure protein, ORF2. Only three of the five identified genogroups have been found to be pathogenic to humans. The virus is stable in the environment and is readily spread from person to person and via contact with contaminated surfaces, as well as via contaminated water or consumption of uncooked or lightly cooked foods. NoV is the most common cause of infectious gastroenteritis in humans (EFSA, 2011) and is the most commonly identified cause of foodborne viral illness (EFSA, 2010). In 2014, viruses were found to have overtaken *Salmonella* as a cause of foodborne outbreaks in the EU, causing 20.4% of all foodborne outbreaks (EFSA and ECDC, 2015). Of the cases reported with strong evidence, the most commonly implicated food vehicle was 'crustaceans, shellfish, molluscs and products thereof' (44.7% of outbreaks) whilst the categories 'fruit' and 'berries and juices' together were implicated in just over 10% of outbreaks. The most implicated causative virus was 'calicivirus – norovirus', which was identified as the cause in 89% of viral foodborne outbreaks categorised as strong-evidence (EFSA and ECDC, 2015).

Of the known genogroups, three are pathogenic to humans, and of these more than 20 genotypes have been identified (FSA, 2015). There is not thought to be cross-immunity between genotypes, so people can fall ill multiple times in a season if more than one genotype is circulating in the community. However, there is some evidence to suggest that there may be complex patterns of cross-immunity in humans (Cannon *et al.*, 2009). Although the majority of outbreaks in the community

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occur in winter, some outbreaks of illness associated with food products have occurred in summer and a large proportion of foodborne illnesses go unreported.

Previously identified research priorities presented to the breakout group were:

- A. Estimate the contribution of foodborne transmission (including food handlers) to the burden of disease and identify the highest risk foods;
- B. Structured survey to estimate the prevalence of NoV in fruit and vegetables (considering infectivity);
- C. Quantification and molecular characterisation of virus contamination in foodstuffs on the European market;
- D. Better understanding of asymptomatic carriage and shedding of NoV in the community and by food handlers.

The group did not feel the list of pre-identified research priorities was the most appropriate one for their area and set about identifying a new list. They were advised to keep the list to no more than six and thus replaced the one provided with the list shown in Table 1. The criteria were ranked using the medians, hence where medians were the same the sum of the rank was divided by the number of questions with the same median value. Thus the criterion ranks were not directly comparable to the overall rank.

Although all six research questions were deemed important, the group agreed that the top four of these in order of importance were B, C, D, and F. This did not strictly reflect the research priorities as identified in the ranking table, which identified A as the number four priority in terms of scoring. This may have been an oversight, however the group emphasised the importance of innovative approaches during discussions and this may have driven the choice.

Research Question	Research priority	Ranking criterion: public health impact	Ranking criterion: feasibility	Ranking criterion: innovation	Rank overall
В	What drives and defines norovirus susceptibility and vulnerability?	1.5	4	3	1
D	What is the impact of asymptomatic carriage and shedding of norovirus in the community and by food handlers?	3.5	2.5	5	2
С	How does finding norovirus in foodstuff relate to public health risk?	3.5	2.5	3	3
A	What are the trends of norovirus source attribution and disease burden, building on what has been done on the WHO report (FAO/WHO, 2008)?	1.5	1	6	4
F	Where are candidate norovirus vaccines likely to have the biggest impact and who do you vaccinate?	5	5	1	5
E	Are there non-human reservoirs and do they drive molecular epidemiology of norovirus?	6	6	3	6

Table 1: Ranked list of research priorities for Group 1

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The final rank was further discussed within the group and question A (how much of the NoV disease burden is due to foodborne illness) was considered to be an overarching question. This question has remained an important question which has been identified as a concern in previous reviews (EFSA, 2011; FSA, 2015). As a consequence, the group considered questions B, C, D and F as the short list, question A is considered addressed from a different angle in question C, which asks how the presence of NoV in foodstuff relates to public health risk.

The rationale offered by the group was:

- a) Need for robust understanding of the baseline disease burden to underpin research questions;
- b) Research questions should address areas for high public health impact to reduce disease burden;
- c) The need for innovative approaches;
- d) Research questions are cross cutting within this theme (epidemiology) and between other themes: methods and control options.

3.2. Breakout Session 2 – Epidemiology and public health impact of hepatitis A virus

Facilitator: Jane Richardson Rapporteur: Mariam Orme

HAV is a single-stranded RNA virus in the family *Picornaviridae*. Only a single serotype of HAV has been identified. The virus is transmitted from person to person via the faecal-oral route or through contact with contaminated food or water (Pinto *et al.*, 2009). HAV is able to persist for long periods in the environment (Sobsey *et al.*, 1988).

Illness in areas with low prevalence is often associated with travel or with consumption of contaminated foods such as bivalve molluscs, salad crops and soft fruits, all of which have been associated with foodborne HAV outbreaks (EFSA, 2011).

The previously identified research questions presented to the breakout group were:

- A. Estimate the contribution of foodborne transmission to the burden of disease and identify the highest risk foods;
- B. Structured survey to estimate the prevalence of HAV in fruit and vegetables (considering infectivity);
- C. Quantification and molecular characterisation of virus contamination in foodstuffs on the European market;
- D. Identify high-risk groups in Europe with regard to prevalence, transmission, participation in seasonal food production activities, and vaccination strategies.

After discussion the group split research question A in two, one area focusing on the contribution of foodborne transmission to the burden of disease and a new area developing risk profiles for food categories considering aspects of both production and processing. The group proposed that shellfish should also be included in the survey (research question B) and that a molecular characterisation project should also include clinical HAV isolates to support source attribution (research question C). Research question D was split into two different areas exploring behavioral practices of food handlers and the effectiveness of interventions aimed at food handlers. In addition, network analysis was proposed as an alternative method to better understand foodborne transmission of HAV. A summary of the individual ranking scores for the research areas is shown in Table 2.

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Research Question	Research priority	Ranking criterion: public health impact	Ranking criterion: feasibility	Rank overall
Ι	What is the contribution of foodborne transmission to the burden of disease in Europe?	1	2	1
А	Risk profiling for food categories, production systems, and processing.	3	1	2
С	Molecular characterisation of virus isolates in food stuffs and clinical samples, including characterisation of antigenic variants.	2	5	3
В	Survey with quantification of HAV in fruit, vegetables and shellfish.	4	4	4
E	Evaluation of screening and vaccination of food handlers.	5	6	5
Н	Network analysis – trade volumes considering endemicity and genotype in country of origin.	7	3	6
F	Seasonal workers: hygiene practices, outreach, living conditions.	6	7	7

Table 2:Ranked list of research priorities for Group 2

After scoring, the top research priorities identified by this group in order of importance were I, A, and C. The group acknowledged that the survey data would be needed to support the risk profiling activity and consequently amend the research question A to the following *Survey (quantification of HAV) to refine risk profiling for food categories, production systems and processing.*

The justification for the top priority research questions is the following:

I. There is a strong suspicion that foodborne transmission of HAV is becoming an increasing problem, which needs to be quantified in order to demonstrate the extent of the problem;

A. High risk food categories, such as soft fruits and seafood, are well known but risk profiling requires a more detailed characterisation that considers production and processing of the different food categories and survey data with quantification of viral contamination and detailed descriptions of food samples would support this the refinement of food risk profiles.;

C. Molecular characterisation of HAV is needed to support to outbreak investigations and to provide insight into viral diversity (spatially and temporally).

3.3. Breakout Session 3 – Epidemiology and public health impact of hepatitis E virus

Facilitator: Marios Georgiadis

Rapporteur: Jesus Alvarez-Pinera/Kasia Kazimierczak

Foodborne infections of HEV are of increasing concern in the UK, as well as across Europe, as the numbers of reported infections appears to be rising. Studies of seroprevalence in the UK indicate as many as 100,000 infections annually in England alone, the majority of which are asymptomatic or unrecognised (Hewitt *et al.*, 2014). HEV is endemic in much of the world, especially in tropical and subtropical regions, and of the four genotypes two (genotypes 3 and 4) are zoonotic and carried by swine. HEV is of particular concern due to its ability to cause severe illness. Although overall,

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mortality is well below 5%, infection in pregnancy results in increased risk of miscarriage, stillbirth and increased mortality and morbidity in neonates.

Recently, cases of potentially foodborne HEV infections have been reported and HEV RNA has been detected in in the pork food chain across the world, suggesting the virus may be commonly carried by swine world-wide. Some evidence has been found of transmission via pork, particularly raw or lightly cooked pork products. However, the role of pork consumption in transmission of this virus is still not fully understood.

In the UK, the incidence of HEV infection has increased since 2004, and the majority of the 579 confirmed cases were not associated with travel to HEV endemic areas (FSA, 2015). The majority of these were in men over 60 years of age. Public Health England found that in England and Wales, infection with locally-acquired hepatitis E was associated with the consumption of processed pork products (Said *et al.*, 2013).

The previously identified research questions presented to the breakout group were:

- A. Epidemiological studies to identify sources, risk factors and the role of the food chain in transmission;
- B. A structured survey on contamination in pork products across the retail sector;
- C. Comparative virus phylogenies in human and pig populations in Member States;
- D. Association between shellfish consumption and infection, potential hazard associated with pig farm effluents impacting shellfish production areas.

As with the previous groups, the list provided was amended and expanded to give what the group felt was a more relevant list of research priorities. The final list of research questions and their chosen priorities is given in Table 3.

Research Question	Research priority	Ranking criterion: public health impact	Ranking criterion: feasibility	Rank overall
с	Comparative virus phylogenies in human and pig populations, food products and production chains, in Member States.	3	1	1
E	What is the burden of hepatitis E in human populations in Europe?	1	2.5	2
A	Epidemiological studies to identify sources (including shellfish and environmental sources), risk factors and the role of the food chain in transmission.	2	4	3
В	A structured survey on contamination in pork products across the retail sector.	4	2.5	4
F	Mapping of pig-derived commodities and their uses, and potential related risks.	5	5	5
D	Potential environmental hazards associated with pig farm effluents, use of pig manure for agriculture and waste water treatment plants impacting shellfish and fresh produce production areas.	6	6	6

Table 3: Ranked list of research priorities for Group 3

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After scoring, the group chose C and E as the top two priorities resulting from the exercise.

The group identified the following rationale for these two proposals.

- a) There is a need to understand the links between animal herds, pork products and human cases of disease. Identifying the source of HEV in Europe. Work already underway to gather data across Europe (HEV net);
- b) We need to develop a better understanding of the burden of HEV in the EU as we do not have a full picture at the moment. This might be difficult to do as a pan European study, but selected countries could contribute as surveillance is already in place. Encourage others to start data gathering.

3.4. Breakout Session 4 – Methods for norovirus and hepatitis A virus in the food chain

Facilitator: Olaf Mosbach-Schulz Rapporteur: Michelle Price-Hayward/ Fraeya Whiffin

NoV is a non-enveloped, single-stranded RNA virus. It is characterised into five genogroups only three of which have been shown to infect humans. These genogroups can be further divided into over 20 genotypes, of which genotype GII-4 has been the predominant source of outbreaks over the past 20 years (FSA, 2015).

Hepatitis A is also a non-enveloped, singled-stranded RNA virus of similar size and structure to NoV. Sequence variation has permitted classification into genotypes, at least five of which are associated with human infections.

Both viruses are extremely stable and can persist for long periods in the environment. Both are inactivated by heat treatment but may remain viable at lower levels of heat ($<85^{\circ}C$).

NoV cannot be cultivated in cell culture, and requires direct detection via electron microscopy, enzyme immunoassays, or reverse transcription polymerase chain reaction (RT-PCR).

HAV can be cultured and can be detected via detection of HAV-specific antibodies in sera or via RT-PCR. Understanding viral infectivity in the food chain is reliant upon developing methods for quantification of virus and particularly for detection of infectious particles.

These two viruses were considered together in a combined breakout session on methodologies due to the similarities between the molecular detection methods used for the two viruses.

The need for harmonised methods for detection of NoV and HAV in food has been previously identified, and a standardised method for the detection and quantification of NoV and HAV has been developed (Lees, 2010).

At the beginning of the elicitation, the group discussed the criteria for the ranking and concluded on the following specifications:

a) Criterion "Impact on Public Health in Europe"

Methods for NoV and HAV in the food chain:

- are necessary:
 - to assess outbreaks;
 - to evaluate measures;
 - to understand the situation;
 - to understand the epidemiology;

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 \circ are in between epidemiology and control;

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- are applicable:
 - on main food items / on regional differences;
 - on many items / global contaminations;
- $\circ \quad$ are necessary to understand the behavior of the virus.
- b) Criterion "Feasibility of implementation":

Methods for NoV and HAV in the food chain:

- can go into legislation;
- have globally good implementation;
- are usable by industry;
- are interpretable:
 - for validation and control;
 - on "positive" and "negative" results.
- are applicable given that they are:
 - fast;
 - cheap / frequent.
- fit well on the one-health approach.

The third criterion "Innovation" was not discussed or used by the group. The initial research priorities given to the group were discussed, modified, combined and amended.

After reviewing the complete list, the group concluded that one research question was not a methodology issue and it was therefore removed from further evaluation.

After initial ranking, the results were presented to the group and an intensive discussion followed. Finally, the group concluded on the rankings shown in Table 4.

Table 4: Ranked list of research priorities for Group 4

Research Question	Research priority	Ranking criterion: public health impact	Ranking criterion: feasibility	Rank overall
В	Methods to evaluate infectivity in control measures and food samples.	1	3	1
С	Develop alternative extraction methods to increase existing sensitivity,	2	2	2
F	Develop new sensitive detection method for other matrices, food and environmental samples.	3	1	3
Н	Harmonisation on interpretation on positive/negative results.	7	5	4
G	Standardisation on typing methods across different sampling types.	6	4	5
A	Developing whole genome sequencing (WGS) methodology.	5	6	6
E	Develop method/assay for culture of norovirus.	4	7	7
-	ethods to establish infectious dose in different food Not evaluated			

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The rationale provided by the group for their rankings were:

- a) Methods to evaluate infectivity are available, but not specifically for food samples;
- b) Improved extraction methods are especially required for detection in fruits and vegetables;
- c) Improved methods are needed for detection and enumeration from surfaces such as carpet / upholstery, and potentially from air;
- d) Clarity is needed to ensure test results are fit and used appropriately for enforcement, policy advice;
- e) There is a need to harmonise the genome regions targeted, as these are currently different for clinical vs food samples and means the two cannot be easily compared or correlated;
- f) Standardisation of WGS is a long-term aim, and may take many years, especially when considering clinical vs food samples;
- g) A method of culture for NoV would very useful, but was not considered very feasible in the near to medium term.

3.5. Breakout Session 5 – Methods for hepatitis E virus in the food chain

Facilitator: Federica Barrucci Rapporteur: Kara Thomas/Giorgia Albieri

HEV is a non-enveloped single-stranded RNA virus with morphology similar to calicivirus. There are four genotypes, with two of them causing disease in humans. HEV can be detected using a number of different serological assays. However, RT-PCR is increasingly used for detection. Although there is no formal international standard as yet for the detection of HEV in food products, a standardised real-time PCR assay has been used for detection in various foods such as pork products (DiBartolo *et al.*, 2012), shellfish (Diez-Valcarce *et al.*, 2012) and leafy vegetables (Kokkinos *et al.*, 2012). There has also been limited progress in development of a cell-culture assay for HEV (Okamoto, 2013).

The previously identified research priorities presented to the breakout group were:

- A. Establish reliable whole genome sequencing methods to support outbreak investigations;
- B. Develop standardised method for assessment of HEV infectivity in different food samples to inform surveys and apply to routine monitoring ;
- C. Develop ISO standard method for detection of HEV in foodstuffs (including pork products);
- D. Develop method for extraction and concentration of HEV from meat matrices;
- E. Develop a PCR method for detection/quantification of HEV in shellfish.

This group used two assessment criteria, impact on public health in Europe and feasibility both in terms of implementation and getting a desired outcome. The group took the opportunity to revise the list, refining the descriptions of priorities to ensure that there was clear distinction between the need for development of methods for the different types of matrices, keeping it to six priorities as listed below.

Priorities following discussion and ranking were:

- A. Development of a quick and cheap assay for genetic strain characterisation of HEV to be obtained with WGS;
- B. Development and validation of direct and indirect methods for assessment of HEV infectivity;
- C. Development of standard methods and ISO methods for detection of HEV in meat and meat products;

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- D. Development of standard methods and ISO method for detection of HEV in other matrices;
- E. Development of standard methods for extraction and detection of HEV in environmental samples;
- F. Standardised Serologic methods to detect HEV antibodies in pigs and humans.

Table 5: Ranked list of research priorities for Group 5

Research Question	Research priority	Ranking criterion: public health impact	Ranking criterion: feasibility	Rank overall
А	Development of a quick and cheap assay for genetic strain characterisation of HEV to be obtained with WGS.	1	1	1
В	Development and validation of direct and indirect methods for assessment of HEV infectivity.	2	2	2
С	Development of standard methods and ISO methods for detection of HEV in meat and meat products.	3	3	3
D	Development of standard methods and ISO method for detection of HEV in other matrices.	4	4	4
E	Development of standard methods for extraction and detection of HEV in environmental samples.	5	5	5
F	Standardised Serologic methods to detect HEV antibodies in pigs and humans.	6	6	6

The following key points were noted in the discussion around the revision and prioritisation of the list of research questions:

- a) The group discussed what methods were used for finding an organism in a sample such as food; and quantify the risk for assessment or to assess infectivity;
- b) WGS was considered a cheap and rapid method; however, the importance of data validation and data sharing was noted, as well as the need to provide a platform for data input;
- c) The group noted challenges with assessing infectivity due to the difficulty in culturing the virus, which impacted the feasibility of implementation;
- d) Different methods are dependent on the different foodstuffs being tested.

3.6. Breakout Session 6– Control options for norovirus and hepatitis A virus in the food chain

Facilitator: Marta Hugas Rapporteur: David Alexander/Jill Wilson

In light of the presence of NoV and HAV in the food chain, methods of control are an important means of ensuring that consumers are presented with safe foods. In shellfish, the risk is largely controlled at primary production and through post-harvest controls. In the case of both viruses, the main route of foodborne contamination is through faecal material, either via exposure to untreated or partially-treated sewage or sewage sludge. However, a significant source of foodborne NoV and HAV outbreaks remains food handlers themselves.

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Outbreaks of viral gastroenteritis and hepatitis associated with berries and leafy green vegetables have been reported globally. In the US, leafy green vegetables were identified as the source of a larger proportion of foodborne NoV outbreaks than any other commodity (Hall *et al.*, 2012).

Effective control of both viruses in the food chain will rely on similar means for avoiding faecal contamination of foodstuffs both in production and preparation and therefore these two viruses are considered together for this breakout theme.

The previously identified research priorities presented to the breakout group were:

- A. Virus survival and inactivation methods in different food matrices and in different stages of the food chain;
- B. Develop models to estimate the impact of interventions for reducing NoV in the food chain on the overall incidence of human infection;
- C. Identify/develop better microbiological indicators for viral contamination in foodstuffs;
- D. Effectiveness of processes in the removal/reduction of virus of commercially harvested shellfish to (e.g. depuration, relaying, high pressure, UV, ozone, irradiation, offshore production);
- E. Investigate effective methods of viral decontamination of food products (other than shellfish) by processing;
- F. Appropriate surrogates in other food matrices to help identify suitable control treatments;
- G. Effectiveness of hand washing procedures, agents used and water temperature;
- H. Efficacy of disinfectants on different surfaces (food preparation surfaces, uniforms, kitchens equipment, soft furnishings);
- I. Development of cost-effective methods for treating waste waters to inactivate viruses.

The group used just the two assessment criteria (impact on public health in Europe and feasibility of implementation) and took a markedly different approach to the other groups. By combining and condensing the list of research questions abovementioned into two, broader research theme areas, the group aimed to ensure that the scope did not exclude innovative approaches and the identification of new or emerging risks. The group also did not wish to rule out the use of virus surrogates or tracers. Scoring was undertaken on an interim list where both of these were then put forward as the selected research priorities for the session.

The research priorities agreed after discussions were:

- A. Implementation of advanced methods to identify sources of contamination and prioritising risk factors from the food supply chain for shellfish and produce to inform assessments;
- B. Identification and validation of intervention strategies for decontamination of NoV and HAV at all stages of the food chain for shellfish and produce.

The group wished to reflect back the following points:

- a) There is a need to bring together research from the food and environmental sectors, linking wastewater treatment, prevention and pollution with food safety impacts;
- b) In order to inform consumer advice and guidance to businesses, there is a need to consider social science aspects of the issues to understand behaviours and what interventions would affect behavioural change. For example, improving our understanding of why people do not wash their hands.

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3.7. Breakout Session 7– Control options for hepatitis E virus in the food chain

Facilitator: Ernesto Liebana Rapporteurs: Milen Georgiev/Bobby Kainth

Outbreaks of HEV have been associated with consumption of inadequately cooked pork products. HEV has been detected in swine herds, abattoirs, processing facilities and in retail product, such as pig livers. Infected pigs do not normally show signs of disease, making it difficult to control for the disease in the production environment. Currently, there are no official controls associated with HEV in pork or pork products. Therefore, given the severity of disease and increasing incidence, a discussion on potential methods of control is both timely and necessary.

The research questions presented to the breakout group were:

- A. Heat inactivation of HEV in naturally contaminated raw, rare and ready-to-eat pork products (considering infectivity);
- B. Investigate effective methods of viral decontamination of food products by processing;
- C. Develop model to estimate the impact of interventions on incidence of viral infections;
- D. Develop microbiological indicators for viral contamination in foodstuffs;
- E. Effect of curing and/or fermentation of pork products on HEV infectivity.

The group felt that the list of research priorities provided in advance of the workshop focused on the food aspects and did not consider the entire food chain or 'one health' approaches that could be useful in addressing HEV risks. Discussions resulted in a complete rewrite of research priorities, with the group agreeing on list below:

- A) Dynamics of HEV in the pig population (in particular how this is affected by husbandry practices)
- B) Development of HEV vaccine intervention strategies on farms (pigs), including development of vaccines
- C) Development of conceptual models of HEV heat inactivation, and validation in foods
- D) Effect on non-thermal processes (e.g. curing, fermentation, etc.) of pork products on HEV infectivity
- E) Development of human exposure assessment/dose response models for HEV
- F) Identification and management (including vaccination, treatment) of human at-risk populations (for HEV)

The group felt strongly that there was a need to understand what was happening at farm level to ensure that some control of the virus problem in primary production could be addressed. Their priorities are given in Table 6.

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Research Question	Research priority	Ranking criterion: public health impact	Ranking criterion: feasibility	Rank overall
F	Identification and management (including vaccination, treatment) of human at risk populations (for HEV).	1	3	1
A	Dynamics of HEV in the pig population (in particular how this is affected by husbandry practices).	2	1	2
С	Development of conceptual models of HEV heat inactivation, and validation in foods.	5	2	3
В	Development of HEV vaccine intervention strategies on farms (pigs), including development of vaccines.	3	6	4
E	Development of human exposure assessment/dose response models for HEV.	4	4	5
D	Effect on non-thermal processes (e.g. curing, fermentation etc.) of pork products on HEV infectivity.	6	5	6

Table 6: Ranked list of research priorities for Group 7

The group provided the following justification for their top three ranked priorities:

- 1) Targeting the 'at-risk' population would solve the large proportion of relevant human cases and would provide the best approach in terms of cost-benefit. This would have the added value of tapping into existing initiatives such as blood screening.
- 2) Targeting the main reservoir of HEV (pigs) would focus on the origin of the problem and reduce the risk later in the food chain. Prevention is better than cure! This could also provide controls that impact on other transmission routes such as environmental contamination. It would allow for the classification of import-export markets and provide a basis for advice to the industry on biosecurity and husbandry practices.
- 3) Will allow provision of concrete advice to both consumers and industry on cooking processed pork products, such as sausages, and will offer a potential control method to protect consumers. Targeted cooking advice could be provided to 'at-risk' groups. The data generated could feed into further exposure assessments and although the model would initially be targeted at pork products, it could potentially be refined to apply to other food products such as shellfish. An added benefit would be potential for protection against other, as yet unidentified, zoonoses.

3.8. Plenary Presentation and Voting

After the conclusion of the breakout discussions, each group briefly presented their conclusions to the full workshop. To avoid confusion, each of the research priorities recommended from the groups was given a sequential number to replace the letter designations. The full numbered list of recommendations is given in Annex D for reference.

The top priorities from each breakout session were placed on posters and displayed around the plenary area for individuals to vote for what they felt were the top three priorities. Delegates were given three colour-coded stickers with which to vote with an instruction that they could not vote for any of the priorities from their own session.

The results of the vote were counted and the top ten presented at the beginning of the plenary session on the next day. The results were as follows:

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- No.22 Group 5: Development and validation of direct and indirect methods for assessment of HEV infectivity (28 votes);
- 2. No.3 Group 1: How does finding NoV in foodstuffs relate to public health risk? (27 votes);
- No.14 Group 4: Methods to evaluate infectivity of NoV and HAV in control measures and food samples (26 votes);
- No.23 Group 5: Development of standard methods and ISO methods for detection of HEV in meat and meat products (24 votes);
- 5. No.13 Group 3: What is the burden of HEV in human populations in Europe (23 votes);
- No.24 Group 6: (NoV and HAV) Implementation of advanced methods to identify sources of contamination and prioritising risk factors from the food supply chain for shellfish & produce to inform risk assessment (22 votes);
- No.25 Group 6: Identification and validation of intervention strategies for decontamination of NoV and HAV at all stages of the food chain for shell fish & produce (14 votes);
- 8. No.12 Group 3: Comparative HEV virus phylogenies in human and pig populations, food products and production chains, in Member States **(13 votes)**;
- No.27 Group 7: Development of conceptual models of HEV heat inactivation, and validation in foods (12 votes);
- 10. No.26 Group 7: Dynamics of HEV in the pig population (in particular how this is affected by husbandry practices) **(11 votes)**.

Of the top ten research questions above, six specifically addressed issues related to HEV. This is in large part due to the less developed state of knowledge around the epidemiology, detection methodology and control of HEV compared with the other two viruses. However, it also reflects concern about the increasing incidence of foodborne HEV infection.

After presentation of the poster results, a printed table of the breakout session results was provided and each table was asked to score the top five priorities for each virus theme. The results were as follows (number in brackets is the sequential number assigned to each research question after the breakout sessions):

Norovirus top five

- 1. (No.14) Methods to evaluate infectivity in control measures and food samples.
- 2. (No.3) How does finding NoV in foodstuff relate to public health risk?
- 3. (No.24) Implementation of advanced methods to identify sources of contamination and prioritising risk factors from the food supply chain for shell fish & produce to inform risk assessment.
- 4. (No.25) Identification and validation of intervention strategies for decontamination of NoV and HAV at all stages of the food chain for shell fish & produce.
- 5. (No.1) Establishing the baseline: what are the trends of NoV source attribution and foodborne disease burden (establish baseline)?

Hepatitis A virus top five

- 1. (No.24) Implementation of advanced methods to identify sources of contamination and prioritising risk factors from the food supply chain for shell fish & produce to inform risk assessment.
- 2. (No.14) Methods to evaluate infectivity in control measures and food samples.

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- 3. (No.25) Identification and validation of intervention strategies for decontamination of NoV and HAV at all stages of the food chain for shell fish & produce.
- 4. (No.6) Methods to evaluate infectivity in control measures and food samples.
- 5. (No.7) Survey (quantification of HAV) to refine risk profiling for food categories, production systems and processing.

Hepatitis E virus top five

- 1. (No.72) Development and validation of direct and indirect methods for assessment of HEV infectivity.
- 2. (No.61) What is the burden of hepatitis E in human populations in Europe?
- 3. (No.51) Development of standard methods and ISO methods for detection of HEV in meat and meat products.
- 4. (No.45) Comparative virus phylogenies in human and pig populations, food products and production chains, in Member States.
- 5. (No.44) Dynamics of HEV in the pig population (in particular how this is affected by husbandry practices).

Overall scoring

When the poster voting scores and the plenary scoring were combined (see Annex A), the following overall research priorities were identified:

Rank	No.	Total Points	Research priority		
1	14	80	Methods to evaluate HAV and NoV infectivity in control measures and food samples.		
2	3	59	How does finding NoV in foodstuff relate to public health risk?		
3	24	58	Implementation of advanced methods to identify sources of contamination and prioritising risk factors from the food supply chain for shellfish & produce to inform risk assessment.		
4	25	37	Identification and validation of intervention strategies for decontamination of NoV and HAV at all stages of the food chain for shellfish & produce		
5	1	36	Establishing the baseline: what are the trends of NoV source attribution and foodborne disease burden?		
6	22	28	Development and validation of direct and indirect methods for assessment of HEV infectivity.		
7	23	24	Development of standard methods and ISO methods for detection of HEV in meat and meat products.		
8	13	23	What is the burden of Hepatitis E in human populations in Europe?		
9	4	17	What is the impact of asymptomatic carriage and shedding of NoV in the community and by food handlers?		
10	23	13	Development of standard and ISO methods for detecting HEV in meat and meat products		

Closing presentations

Dr. Penny Bramwell of the FSA provided a closing presentation that covered the FSA strategic plan for 2015-2020 and virus research that has been funded by FSA to date, including a research study to develop a behavioral model that outlines how food handlers and management practices affect the risk

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of NoV transmission. Encouragement of wider ownership for food safety by both food business operators and food handling staff and a reduced role for regulators was highlighted. As with other government bodies and research councils, FSA is facing funding limitations and therefore Dr. Bramwell identified other potential means of funding the priorities identified by the workshop, such as through cooperation and partnership working, including a role for industry funding of research.

Luis Vivas-Alegre from DG-RTD presented, via video link, the Horizon 2020 Societal Challenge 2 work programme, as well as identifying other potential routes for funding work in food safety, such as the Marie Sklodowska-Curie Actions and the Small and Medium-sized Enterprise Instrument.

4. Conclusions

The following priorities were identified through the expert elicitation exercise as the group consensus on research priorities in the area of foodborne virus research;

<u>Priority 1</u>: development and validation of direct and indirect methods for assessment of HEV infectivity

HEV is a growing concern in a number of member states as well as in the UK, however there has been to date little communication and linkage between clinicians, epidemiologists, food processors and primary producers to discover how this virus travels from farm to fork and to address the best ways to reduce the burden of human disease.

Although there has been some limited progress on development of culture methods for hepatitis E, there is still much to be done to ensure that infectivity can be determined in a reliable and reproducible manner. This will aid quantification of virus in various food products and allow a more accurate assessment of risk to consumers.

<u>Priority 2</u>: establishing how the detection of norovirus in foodstuffs relates to public health risks

NoV has been detected at low levels in many food products, but there is a lack of clarity regarding how this relates to risk of illness in consumers. At the EU level, it is not known how much disease caused by NoV can be attributed to foodborne spread. Studies in some countries suggest this could be significant. However, the relative contributions of shellfish, fresh produce, food handlers (including asymptomatic shedders) and the food handling environment have not been determined. Current EU surveillance for foodborne NoV illness does not capture dispersed outbreaks very efficiently, and there is clear evidence of significant underreporting of foodborne NoV outbreaks. This priority is related to (dependent upon) development of methods to detect viable virus and then establishing how many viable particles are sufficient to cause illness from consumption of foodstuffs.

<u>Priority 3</u>: methods to evaluate norovirus and hepatitis A infectivity in control measures and food samples

Currently, the methods available to detect NoV and HAV in food samples are not able to discriminate between infectious and non-infectious virus. More studies are needed on the relation between detection of virus genomic copies by PCR in food and probability of causing disease. For this purpose, guidance for outbreak investigation for foodborne virus-related outbreaks could be drawn up to generate the type of data needed for quantitative microbial risk assessment (QMRA). Depending on the efficacy of these tests and baseline levels found in foods, this could then be translated into control measures. This priority is effectively about developing methods for detection of viable virus.

<u>Priority 4</u>: development of standard methods and ISO methods for detection of HEV in meat and meat products

Although HEV can be detected in foods using similar methods to those used for HAV and NoV, these methods are not as far along in development and require validation and standardization in the food products at greatest risk of transmitting HEV: meat and meat products, particularly pork. Currently there is no standard or ISO method specifically for HEV detection in meat. Developing this standard

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will allow for greater confidence in the accuracy and consistency of testing for HEV in meat and allow for results to be compared across laboratories and countries. This will be a necessary step to establishing what the baseline is with regard to contamination of meat products and then beginning to address how to control the risk of infection from meat products.

<u>Priority 5</u>: establishing the burden of hepatitis E in human populations in Europe

It is not currently known how many people have been exposed to hepatitis E and how many subclinical cases occur every year. Population-level estimates of incidence, source attribution, and clinical impact of HEV in humans in general, and in specific risk groups such as the elderly and the immunocompromised, are needed to determine the burden of disease, including foodborne illness.

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Abbreviations

DG RTD	Director General Research and Innovation (European Commission)
DNA	Deoxyribonucleic acid
EFSA	European Food Safety Authority
EKE	Expert knowledge elicitation
EIA	Enzyme immuno-assay
EM	Electron microscopy
FSA	Food Standards Agency
HAV	Hepatitis A virus
HEV	Hepatitis E virus
NoV	Norovirus
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
SS	Single-stranded
WGS	Whole genome sequencing
WHO/FAO	World Health Organisation Food and Agriculture Organisation

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Annex A – Table of overall priorities resulting from the workshop

Group	No.	Group priority	Overall priority	Research Priority	
1	1	1	28	Establishing the baseline: What are the trends of NoV source attribution and foodborne disease burden? (establish baseline)	
	2	2	21	What drives and defines NoV susceptibility and vulnerability?	
	3	3	2	How does finding NoV in foodstuff relate to public health risk?	
	4	4	12	What is the impact of asymptomatic carriage and shedding of NoV in the community and by food handlers?	
	5	5	22	Where are candidate NoV vaccines likely to have the biggest impact, who do you vaccinate?	
2	6	1	16	What is the contribution of foodborne transmission to the burden of disease in Europe?	
	7	2	13	Survey (quantification of HAV) to refine risk profiling for food categories, production systems and processing	
	8	3	23	Molecular characterization of virus isolates in foodstuffs and clinical samples	
	9	4	24	Evaluation of screening and vaccination of food handlers	
	10	5	18	Network analysis of trade volumes vs origin (endemicity + genotype)	
	11	6	25	Seasonal workers' hygiene practices and outreach	
3	12	1	8	Comparative virus phylogenies in human and pig populations, food products and production chains, in Member States	
	13	2	5	What is the burden of hepatitis E in human populations in Europe?	
4	14	1	3	Methods to evaluate infectivity in control measures and food samples	
	15	2	19	Develop alternative extraction methods to increase existing test sensitivity	
	16	3	29	Develop new sensitive detection method for other matrices, food and environmental samples	
	17	4	14	Harmonisation on interpretation of positive/negative results	
	18	5	30	Standardisation of typing methods across different sample types (clinical vs food)	
	19	6	17	Developing WGS methodology	

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Group	No.	Group priority	Overall priority	Research Priority		
	20	7	26	Develop a method/assay for culture of NoV		
5	21	1	15	Development of a quick and cheap assay for genetic strain characterization of HEV to be obtained with WGS		
	22	2	1	Development and validation of direct and indirect methods for assessment of HEV infectivity		
	23	3	4	Development of standard methods and ISO methods for detection of HEV in meat and meat products		
6	24	1	6	Implementation of advanced methods to identify sources of contamination and prioritizing risk factors from the food supply chain for shellfish and produce to inform risk assessment		
	25	2	7	Identification and validation of intervention strategies for decontamination of NoV and HAV at all stages of the food chain for shellfish and produce		
7	26	1	10	Dynamics of HEV in the pig population (in particular how this is affected by husbandry practices)		
	27	2	9	Development of conceptual models of HEV heat inactivation, and validation in foods		
	28	3	11	Identification and management (including vaccination, treatment) of human at-risk populations for HEV		
	29	4	20	Development of HEV vaccine intervention strategies on farm for pigs		
	30	5	27	Effect of non-thermal processing (e.g. slicing, fermentation, etc.) of pork products on HEV infectivity		

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Annex B – Statistical output from the group research ranking exercise

Below is the graphical output from the ranking exercise as described in Section 2 (Data and methodologies) of the report. The relevant research questions for each group can be cross-referenced in Section 3.1-3.7. In the tables of rankings for each group below, it should be noted that the lower the score the higher the ranking of the particular research question.

Group 1



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Criteria and Overall Rankings - Group 1

Research Questions	Criteria 1 (W = 1)	Criteria 2 (W = 1)	Criteria 3 (W = 1)	Overall Ranking
RQB	3.00	3.53	2.83	3.12
RQD	2.90	2.73	3.87	3.17
RQC	3.30	3.10	3.50	3.30
RQA	2.97	2.60	4.60	3.39
RQF	3.90	3.80	2.77	3.49
RQE	4.93	5.23	3.43	4.53

Group 2



Criteria 1 - Group 2



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Criteria and Overall Rankings - Group 2

	Criteria 1 (W = 1)	Criteria 2 (W = 1)	Overall Ranking
RQI	3.14	2.64	2.89
RQA	3.64	2.57	3.11
RQC	3.29	4.36	3.82
RQB	3.79	4.29	4.04
RQE	4.00	5.07	4.54
RQH	5.29	3.93	4.61
RQF	4.86	5.14	5.00

Group 3





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Criteria and Overall Rankings - Group 3

	Criteria 1 (W = 1)	Criteria 2 (W = 1)	Overall Ranking
RQC	2.96	1.75	2.36
RQE	1.71	3.14	2.43
RQA	2.75	3.61	3.18
RQB	4.29	3.14	3.71
RQF	4.39	4.54	4.46
RQD	4.89	4.82	4.86

Group 4





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Criteria and Overall Ranking - Group 4

	Criteria 1 (W = 1)	Criteria 2 (W = 1)	Overall Ranking
RQB	2.00	3.75	2.88
RQC	3.29	3.04	3.16
RQF	3.93	2.75	3.34
RQH	4.54	3.79	4.16
RQG	5.00	3.46	4.23
RQA	5.04	5.00	5.02
RQE	4.21	6.21	5.21

Group 5





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Criteria	and C	Overall	Rankin	1g - G	iroup 5
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	Criteria 1 (W = 1)	Criteria 2 (W = 1)	Overall Ranking
RQA	1.56	1.18	1.37
RQB	2.22	2.22	2.22
RQC	3.07	3.34	3.20
RQD	3.79	3.96	3.88
RQE	4.85	4.79	4.82
RQF	5.51	5.51	5.51

Group 6





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Criteria	and	Overall	Ranking	- Group	p 6
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	Criteria 1 (W = 1)	Criteria 2 (W = 1)	Overall Ranking
RQI	3.19	3.56	3.38
RQA	3.19	3.59	3.39
RQH	4.34	2.78	3.56
RQJ	3.47	4.34	3.91
RQB	4.12	4.03	4.08
RQC	4.81	4.69	4.75
RQF	4.88	5.00	4.94

Group 7







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Criteria and Overall Ranking - Group 7

	Criteria 1 (W = 1)	Criteria 2 (W = 1)	Overall Ranking
RQF	2.42	3.19	2.81
RQA	3.04	2.85	2.94
RQC	3.96	3.00	3.48
RQB	3.12	4.27	3.69
RQE	3.92	3.73	3.83
RQD	4.54	3.96	4.25

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Annex C – Tables of final plenary voting by virus

Norovirus

Total Scores	36	4	59	17	4	80	6	6	7	2	8	1	58	37
	Group 1				•		Group 4						Group 6	
Research Priorities	1	2	3	4	5	14	15	16	17	18	19	20	24	25
VOTING	0	2	27	8	2	26	3	0	6	0	4	1	22	14
Table One	3		2			5							4	1
Table Two	3		5			4							2	1
Table Three		2	4			4							3	2
Table Four	5			2		3							4	1
Table Five			3			5		2					1	4
Table Six	5		2			3							4	1
Table Seven	3		2	2	2	2							2	2
Table Eight	2		3			5				1				4
Table Nine			4	1		5					2			3
Table Ten			4			5					2		3	1
Table Eleven	5			2		4				1			3	
Table Twelve			3					4	1				5	2
Table Thirteen	5					5							5	
Table Fourteen	5			2		4	3							1

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Hepatitis A Virus

Total Scores	37	32	21	9	7	4	55	10	6	8	0	10	1	58	45
	Group 2							Group 4						Group 6	
Research Priorities	6	7	8	9	10	11	14	15	16	17	18	19	20	24	25
VOTING	4	6	2	1	3	1	26	3	0	6	0	4	1	22	14
Table One	5	2	1											4	3
Table Two	5	3	1				4							1	1
Table Three	5	4		1				3				2			
Table Four	5		4			1								2	3
Table Five	5		3				4		3						
Table Six		5		1			3							3	3
Table Seven	5				4									3	3
Table Eight				1			5			2				3	4
Table Nine			2				5					1		3	4
Table Ten				4					3			1		5	2
Table Eleven		5	3				4							3	
Table Twelve			5									2		4	4
Table Thirteen	3	2					1	4						5	
Table Fourteen		5		1		2	3								4

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Hepatitis E Virus

Total Scores	45	61	7	72	51	44	28	24	3	4	
	Gro	up 3		Group 5			Group 7				
Research Priorities	12	13	21	22	23	26	27	28	29	30	
VOTING	13	23	5	28	24	11	12	9	3	1	
Table One	4			5		2	3			1	
Table Two	1	5		3	4	1	1				
Table Three	2	5		3	4		1				
Table Four		5			3	2	1	4			
Table Five	5			5		5					
Table Six	1	2		3		4		5			
Table Seven		4		1	4	3	3				
Table Eight	3			2		3	2	3		2	
Table Nine		3	2	4	5		1				
Table Ten	4			5	1	3		2			
Table Eleven	3	4		3	3		2				
Table Twelve	5			2	3	4	1				
Table Thirteen	2	5		4		3		1			
Table Fourteen	2	5		4		3	1				

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Annex D – Technical specification for the grant

The workshop deliverables for Cefas, as grant beneficiary, were as follows:

- Participation in meetings of the organising committee via video/teleconference;
- Provision of advice on invitees and contents;
- Provision of advice on organisation of breakout session, including on chairs and rapporteurs;
- Hire of a suitable venue offering a plenary room plus space for up to 7 breakout sessions, including related IT support;
- Arrangement of catering to include coffee/tea breaks, lunch for 2 days and one evening meal;
- Arrangement of a registration website to enable registration of invited attendees;
- Arrangement of travel and accommodation, including transfers, for invited speakers;
- Provision of registration assistance by telephone/email prior to and at the workshop;
- Provision of workshop packs, to include printed material relevant to the workshop, name badges, blank paper and pens;
- Input and support for development of pre-workshop questions for participants;
- Provision of two staff as rapporteurs, who then collated reports from the various breakout sessions and provided input to the wrap up session on the last day;
- Provision of the final workshop report after completion of the workshop.

A timeline of the major milestones in development of the workshop are shown below.

Timeline

•	Formation of scientific organising committee	May 2015
•	Workshop themes agreed	June 2015
•	Cefas contacted for organisation	July 2015
•	Location and venue agreed	October 2015
•	Invited speakers confirmed	October 2015
•	Invitations issued to delegates	November 2015
•	Confirmation of attendance sent	January 2016
•	List of recent research priorities agreed	February 2016
•	Supporting materials sent to delegates	January-February 2016
•	Workshop held	February 2016

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Annex E – List of participants

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