

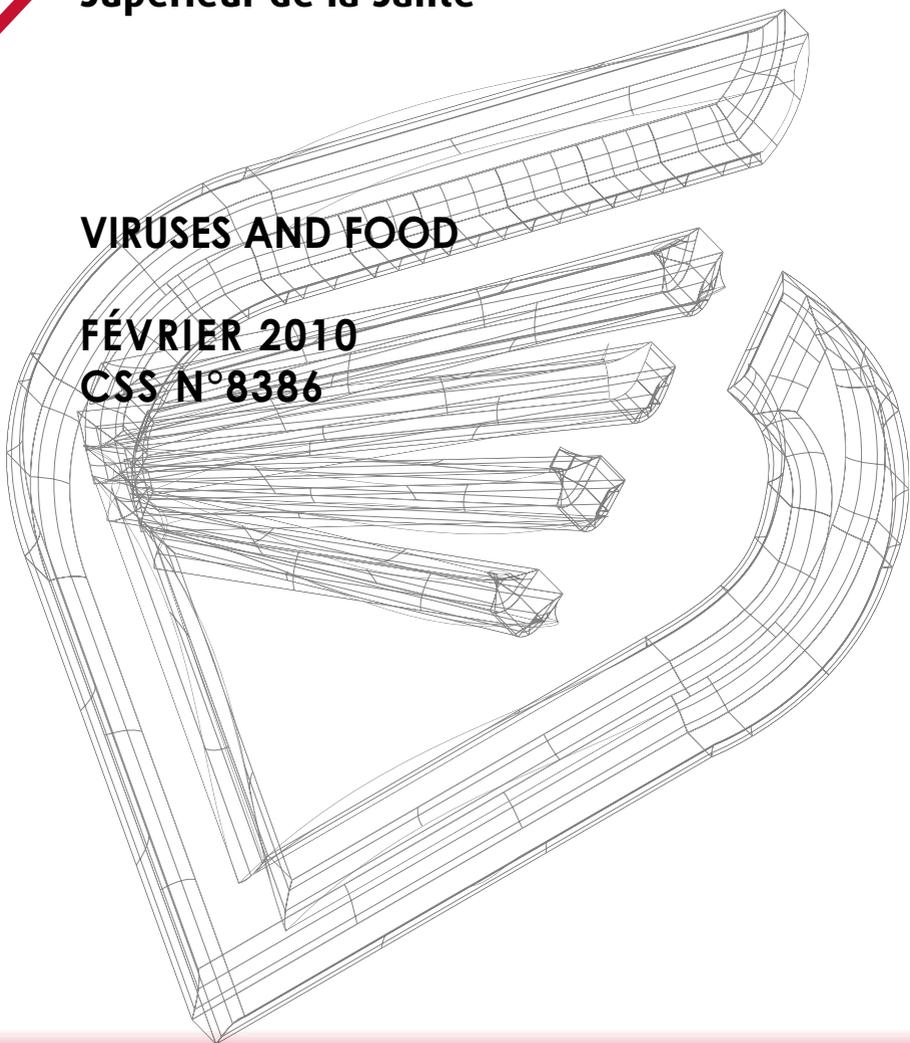


**Conseil
Supérieur de la Santé**

VIRUSES AND FOOD

FÉVRIER 2010

CSS N°8386





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Supérieur de la Santé**

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Veuillez citer cette publication de la façon suivante:
Conseil Supérieur de la Santé. Viruses and Food. Bruxelles: CSS;
2010. Avis n° 8386.

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Numéro de série Dépôt légal: D/2010/7795/7

N° ISBN: 9789490542092

Cette publication ne peut être vendue.

INTRODUCTION

Les virus transmissibles directement via les aliments et l'eau représentent actuellement la source d'un problème majeur de santé publique dans de nombreuses régions du monde. Ces virus se retrouvent dans le tractus gastro-intestinal via ces voies alimentaires directes de transmission et peuvent être à l'origine de gastro-entérites ou d'hépatites. Ce problème est qualifié d'émergent et demande que l'on consacre une attention particulière à l'information des autorités, du consommateur et des secteurs professionnels en ce qui concerne les denrées alimentaires à risque élevé et les méthodes de travail.

En termes d'implication pour la santé publique, il a été jugé utile et opportun que le Conseil Supérieur de la Santé (CSS) – tout en tenant compte de ce qui se déroule actuellement au sein des autres instances scientifiques (nationales et internationales) – puisse dresser un bilan synthétique de la situation au niveau national et proposer des recommandations en la matière.

Un groupe de travail *ad hoc* du CSS (au sein duquel des expertises en microbiologie alimentaire et en virologie sont représentées) a réalisé une revue des informations actuellement disponibles en matière de risque pour l'homme lors de la consommation de denrées alimentaires contaminées par des virus afin d'émettre des recommandations en vue d'un meilleur contrôle des infections virales d'origine alimentaire. La version finalisée de ce texte a ensuite été soumise aux commentaires et à l'approbation du groupe « *Microbiologie des aliments* » du CSS.

Le présent document reprend de manière abrégée les idées principales développées dans le rapport scientifique joint en annexe à cette publication. La démarche générale suivie a été de passer en revue les différents aspects concernés: les caractéristiques des principaux virus liés aux denrées alimentaires (et les questions qu'ils peuvent soulever au sein des autorités compétentes) et que faire pour gérer les épisodes épidémiques (*outbreaks*) de manière appropriée (bases du comportement à adopter en cas d'épidémie, du moins du point de vue de la transmission par le biais des denrées alimentaires). Les informations recueillies se basent sur la littérature scientifique récente.

CONCLUSION

A partir des constats et des observations collationnés dans le rapport scientifique (repris en annexe) et des nombreux points mis en exergue dans ce document, des conclusions orientées santé publique (aspects nationaux et internationaux) ont pu en être tirées.

Bien que seule une faible fraction des cas seulement soit rapportée au système national de surveillance et que même si de nombreux pays ne disposent d'aucun système de surveillance efficace, les données épidémiologiques disponibles et les publications récentes de la littérature montrent que les denrées alimentaires peuvent agir comme vecteur efficace de transmission des virus.

Les virus liés aux denrées alimentaires se transmettent par voie féco-orale. La contamination fécale s'effectue par les professionnels (*food handlers*) manipulant les produits du type « prêts-à-consommer », par l'eau dans laquelle sont élevés des mollusques bivalves et par l'eau utilisée lors de la culture/production des produits frais. Les virus ne se répliquent pas au sein des denrées alimentaires. Cela signifie donc que leur nombre ne dépassera jamais la charge virale initiale. Cette charge virale habituellement faible dans les échantillons analysés permet de comprendre les difficultés concrètes rencontrées pour le diagnostic d'une contamination virale. Les mollusques représentent dans ce même ordre d'idée une source alimentaire à risque dans la mesure où ils s'alimentent par filtration et concentrent donc physiologiquement les particules virales dans leur tractus digestif.

En Belgique, le norovirus (NoV) et le virus de l'hépatite A (HAV) sont les plus importants en termes de prévalence (nombre de cas) et de sévérité des pathologies associées. D'autres virus transmissibles par les denrées alimentaires, d'importance moindre mais également susceptibles d'entrer en ligne de compte sont: le virus de l'hépatite E (HEV), les rotavirus et les sapovirus humains. L'HEV a pu rejoindre ce groupe dans la mesure où il a été récemment détecté en Belgique chez l'homme et le porc. La plupart de ces virus ont une transmission de type féco-orale. En troisième lieu, d'autres virus peuvent également être mentionnés: les astrovirus, les adénovirus de types 40 et 41, les virus Aichi, les entérovirus et le virus de l'encéphalite transmissible par les tiques (TBEV). Certains virus zoonotiques émergents dont on ne peut exclure qu'ils puissent être transmis par les denrées alimentaires (comme p.ex., le virus influenza aviaire H5N1, le virus Nipah, la rage) n'ont pas été repris dans le rapport scientifique dans la mesure où la transmission par les denrées alimentaires est considérée comme négligeable.

Bien que le pathogène viral le plus fréquent, le NoV, n'occasionne que des affections modérées, leur haute incidence illustre bien leur potentiel inhérent à engendrer des épidémies d'origine alimentaire et à très large diffusion internationale. Il existe néanmoins également un risque de diffusion par voie alimentaire d'affections plus graves, telles que l'hépatite.

Des études épidémiologiques de qualité permettront de mettre en évidence toute modification dans les profils épidémiologiques et, particulièrement, l'émergence – ou du moins l'augmentation d'incidence – de virus tels que l'HEV, le virus Aichi ou le TBEV qui se manifeste surtout en Europe du Nord.

La proximité génétique de quelques souches virales animales et humaines soulève des questions au sujet de la potentielle transmission zoonotique ou au sujet de l'aspect « réservoir animal ». Pour mieux comprendre les voies de transmission de ces virus, tels que les norovirus et l'HEV, il est indispensable de pouvoir réaliser le séquençage des souches animales et humaines. Il est dès lors important que des recherches soient réalisées sur des souches virales tant humaines qu'animales.

SITUATION EN BELGIQUE

Selon l'EFSA (*European Food Safety Authority*), les virus transmis par les denrées alimentaires (adenovirus, norovirus, entérovirus, HAV et rotavirus) ont été responsables de 10,2 % des épidémies déclarées en 2006, montrant une augmentation sensible en un an (5,8 % en 2005). En 2006 et 2007, les virus ont été classés en deuxième place après les *Salmonella* dans les causes d'infection d'origine alimentaire. Les norovirus sont la cause la plus fréquente des gastro-entérites non bactériennes (selon le Foodborne Viruses in Europe network, FBEV, 1995-2000) et représentent plus de 60 % des épidémies causées par des virus transmis par les aliments (196/315 épidémies, soit 6.006 cas individuels en 2005) (rapport de l'EFSA, 2007).

En Belgique, 20 à 50 % des causes d'épidémies d'origine alimentaire sont indéterminées. Cette situation s'est améliorée depuis 2006, à la suite de la mise en œuvre d'une méthode de détection des norovirus dans les denrées alimentaires. Le nombre d'infections à norovirus reste sous-évalué, car la gastro-entérite guérit rapidement et les patients ne consultent par conséquent pas nécessairement un médecin. En outre, peu d'échantillons cliniques sont analysés car leurs coûts ne sont pas remboursés en Belgique. En 2006, 3 épidémies à norovirus ont été mises en évidence en Belgique. Dès 2007, 10 des 75 épidémies d'origine alimentaire étaient causées par des norovirus, qui représentent depuis lors la première cause de ces épidémies enregistrées dans notre pays, devant les *Salmonella*.

L'infection par le HAV est rapportée régulièrement en Belgique: 194 cas en 2006 et 197 cas en 2007. Quoique la transmission du HAV soit principalement de nature féco-orale, dans certains cas, l'origine suspectée est un membre du personnel infecté par le HAV et manipulant les denrées alimentaires.

Le rotavirus, un autre virus principalement transmis par la voie féco-orale, est également fréquemment rapporté en Belgique (seulement une très faible proportion des 4.194 cas rapportés en 2007 sont d'origine alimentaire). Les sapovirus sont aussi présents en Belgique, en faible incidence par rapport aux norovirus et rotavirus. On ne dispose pas d'information sur l'origine alimentaire des cas d'infections à rotavirus et à sapovirus.

L'infection par le virus HEV a déjà été mise en évidence chez l'homme en Belgique. Une étude récente démontre sa présence également chez le porc dans notre pays. Le lien entre l'infection due à HEV et l'alimentation n'a pas été constaté à ce jour en Belgique.

CONSTATATIONS ET RECOMMANDATIONS

Compte tenu des connaissances scientifiques actuelles dans la littérature sur les virus transmissibles par les denrées alimentaires, de l'état actuel des méthodes analytiques et des données épidémiologiques disponibles, le CSS adresse aux autorités compétentes et aux gestionnaires un ensemble de recommandations relatives à cette problématique de santé publique.

- Les virus d'origine alimentaire sont classés par ordre de priorité en fonction de leur rôle dans les transmissions par les aliments. Les **norovirus** et le **HAV** se situent au **niveau 1**, étant donné qu'il s'agit des virus les plus fréquemment à l'origine d'épisodes épidémiques viraux causés par les aliments. Les sapovirus, HEV et rotavirus se situent au niveau 2. Les virus Aichi, TBEV, astrovirus, adénovirus et entérovirus se classent parmi les virus de niveau 3. En effet, peu d'épisodes épidémiques d'origine alimentaire sont attribués à ces derniers virus.
- Il n'y a guère de certitude quant à la **dose infectieuse des virus d'origine alimentaire**. On estime que cette dose est extrêmement basse, de l'ordre de **10 à 100 particules virales** environ. En outre, les virus d'origine alimentaire sont souvent éliminés à des concentrations extrêmement élevées pouvant atteindre **10⁹ particules virales / gramme de selles**.
- Selon une estimation effectuée à l'aide de techniques sensibles, les norovirus et HAV peuvent être excrétés **pendant plusieurs semaines** après une infection accompagnée ou non de symptômes. Il y a lieu de recommander la mise en place de mesures d'hygiène spéciales pour les personnes travaillant dans le secteur des soins de santé ou dans l'industrie alimentaire qui manipulent ou préparent manuellement les denrées alimentaires. En ce qui concerne les personnes pour lesquelles il a été établi qu'elles excrètent ces virus, il convient de prendre des précautions supplémentaires.
- Les virus d'origine alimentaire sont principalement disséminés par voie féco-orale. La transmission de **personne à personne** est importante et notamment la transmission intrafamiliale; elle est suivie par la transmission par l'intermédiaire **d'aliments contaminés et d'eau potable ou récréative**. Il ne faut pas sous-estimer les sources environnementales à la base des infections virales. Il n'est pas toujours facile de déterminer quelle proportion parmi les maladies infectieuses humaines est imputable à la consommation de denrées alimentaires. Il n'est pas plus évident de déterminer les denrées concernées de manière spécifique.
- **Les légumes et les fruits frais** peuvent être considérés comme des aliments **à risque élevé**. Ils peuvent être contaminés avant la récolte suite à un contact avec des eaux d'irrigation polluées par des matières fécales, avec de l'engrais à base de matières organiques ou par les cueilleurs (lors de la récolte). Il n'y a guère de certitude quant à la proportion dans laquelle ces différentes voies

de transmission possibles sont impliquées. On ne dispose que de peu de données quant à la présence de virus dans les différents types d'eau utilisés dans la production primaire de plantes en Belgique. De plus, les facteurs de risque de contamination découlant du contact entre les eaux d'irrigation polluées et les légumes et fruits frais sont mal connus.

- **Les aliments traités manuellement et destinés à être consommés sans réchauffement préalable, tels que les plats préparés, peuvent représenter un risque élevé** de contamination virale. En raison de la faible dose infectieuse et de l'élimination importante des virus infectieux, il convient d'écarter les travailleurs contaminés actifs dans le secteur alimentaire au moins jusqu'à la fin de la forme aiguë de l'affection. Des mesures d'hygiène complémentaires doivent être prises lors de leur retour de convalescence.
- Les **mollusques** font également partie des **aliments à risque élevé** du fait que ces animaux sont des filtreurs. Les virus sont ainsi filtrés de l'eau polluée environnante, conservés et peuvent même être concentrés dans les tissus digestifs des mollusques bivalves (huîtres, moules, coques, palourdes). Si des mollusques contaminés sont consommés crus ou seulement légèrement cuits (juste jusqu'à ce que les coquilles soient ouvertes), il y a un risque d'infection virale.
- A l'heure actuelle, on ne dispose pas d'évaluation complète des risques posés par les principaux pathogènes viraux, NoV et HAV, dans les aliments à risque précités. Cette évaluation sera difficile à réaliser. Elle nécessite une meilleure compréhension des voies de transmission, de la prévalence, de la persistance et des titres de particules infectieuses de ces virus dans la chaîne alimentaire. De plus, les données quantitatives font actuellement défaut.
- Le critère microbiologique obligatoire (Règlement (CE) N°854/2004 et N°2073/2005) à observer lors de la vérification des mollusques bivalves vivants est basé sur le niveau de l'indicateur bactérien **E. coli** et **nullement sur la présence de virus**. Il faut donc des indicateurs viraux complémentaires qui portent sur la présence de virus pathogènes pour l'homme.
- Contrairement aux bactéries, les virus sont incapables de se développer à l'extérieur de leur hôte et donc dans un milieu de culture. De plus, la plupart des **virus d'origine alimentaire ne peuvent pas être cultivés en laboratoire** au moyen de cultures cellulaires ou présentent une croissance difficile. C'est pourquoi leur détection se fonde sur des **tests de détection moléculaire**. La PCR est la technique par excellence utilisée pour détecter les virus transmis par l'alimentation. La fiabilité des résultats requiert des **contrôles adéquats des tests de détection moléculaires**, notamment un contrôle interne de l'amplification pour vérifier l'absence d'inhibition de la PCR et un contrôle de processus pour vérifier l'efficacité de la préparation de l'échantillon.

- L'absence de méthodes de culture signifie que, même lorsque les virus sont présents en faibles quantités, leur extraction exige préalablement la mise en place de **méthodes adaptées pour préparer**, à partir des aliments, **des échantillons** de petits volumes destinés à la RT-PCR. **Aucune méthode horizontale d'extraction virale** n'est possible. Il semble nécessaire de catégoriser les aliments en fonction de leur composition (par exemple: aliments de type gras, aliments de type aqueux). L'**harmonisation** et la catégorisation sont toujours en cours au niveau européen (CEN) et mondial (Etats-Unis, Canada). Des essais inter-laboratoires de grande envergure sont nécessaires pour sélectionner des méthodes d'extraction virale robustes, simples et fiables.
- Etant donné que la **détection** virale est basée sur la détection moléculaire, celle-ci vise le **génomme viral**. Le test de détection moléculaire montrera la présence/l'absence de copies du génome viral. Un résultat positif indique qu'il y a contamination virale. Par contre, la détection de copies du génome viral par RT-PCR ne signifie pas nécessairement que des particules virales infectieuses sont présentes. Dans cet état des choses, de nouvelles méthodologies de détection permettant **de distinguer les particules virales infectieuses des particules virales non infectieuses** sont requises.
- Afin d'éviter la contamination virale des produits alimentaires, les bonnes pratiques agricoles (BPA), les bonnes pratiques de fabrication (BPF) et les bonnes pratiques d'hygiène alimentaire (BPH) revêtent une importance capitale. Les épisodes épidémiques viraux d'origine alimentaire sont fréquents, ce qui signifie qu'il y a des entorses aux « bonnes pratiques » dans la chaîne alimentaire. Parmi les manquements typiques figurent des irrégularités au niveau de la performance même des mesures préventives et le respect relatif des procédures (par exemple: mauvaises pratiques au niveau du nettoyage, mauvais comportement hygiénique). Le respect des procédures peut être influencé par la prise de conscience et la connaissance des conseils et des procédures, mais aussi par la persistance d'habitudes et de comportements existantes. Le risque de contamination peut être réduit en vaccinant les travailleurs du secteur alimentaire. De tels vaccins sont déjà disponibles pour le HAV et le virus de la poliomyélite. Il n'existe pas de vaccin disponible contre le NoV.
- Les **méthodes de conservation alimentaire** basées sur l'inhibition et l'inactivation de la croissance microbienne doivent être évaluées quant à leur **efficacité** pour réduire/éliminer **les virus d'origine alimentaire**. Trop peu de données concernant la stabilité des virus soumis aux technologies de transformation alimentaire sont actuellement disponibles.
- Des épisodes épidémiques survenus dans des maisons de repos et sur des bateaux de croisière ont été expliqués par la **contamination des surfaces** et des mains du personnel soignant et des travailleurs du secteur alimentaire; ceci montrant la stabilité des virus d'origine alimentaire. Davantage de données quant à l'efficacité des désinfectants (biocides) et des agents nettoyants sont requises.

- Il faut améliorer l'**investigation des épisodes épidémiques d'origine alimentaire**, ce qui impliquera la mise à disposition de ressources suffisantes pour **améliorer le réseau** reliant d'une part le Laboratoire de référence pour les épisodes épidémiques effectuant les analyses des aliments avec d'autre part l'unité d'épidémiologie rassemblant les informations épidémiologiques. Cette démarche permettra de réduire le sous-rapportage des épisodes épidémiques viraux d'origine alimentaire en Belgique.
- Il faut promouvoir l'analyse virale des échantillons cliniques et trouver des sources alternatives de financement. Cela permettra de réduire le sous-rapportage et, de la sorte, d'améliorer les estimations quant à la charge sociale que représentent les maladies virales d'origine alimentaire.
- Les propriétés zoonotiques de même que la présence de **réservoirs animaux pour les virus d'origine alimentaire font toujours l'objet d'études**. A l'heure actuelle, on ne dispose pas de preuves quant au rôle des animaux de rente ou de compagnie dans la chaîne de transmission des norovirus. Toutefois, les HEV sont présents chez les porcs et il convient de déterminer clairement le rôle de ce réservoir. La présence de sapovirus et de virus Aichi chez les animaux de rente constitue un aspect intéressant auquel il convient de prêter une attention particulière.
- Les norovirus ne sont pas connus du grand public en Belgique. Aussi, beaucoup de médecins et de professionnels de la santé occupés dans des institutions semi-fermées – telles que les hôpitaux, les maisons de soins, les centres de jour – ne sont pas informés de l'existence de ce virus. Etant donné que les NoV sont **très contagieux**, ils peuvent se répandre très facilement. Les NoV provoquent des gastroentérites généralement légères mais peuvent également engendrer des **affections sérieuses** chez les personnes sensibles telles que les **jeunes enfants, les personnes âgées, les individus immunodéprimés, ...**
- Il est fortement recommandé de **délivrer des informations** sur les virus d'origine alimentaire (NoV, HAV) aux docteurs en médecine, aux personnes actives dans le secteur des soins de santé, aux personnes travaillant avec des groupes sensibles, aux responsables de systèmes de gestion de la sécurité alimentaire. Il est également recommandé de fournir une formation spécifique et adaptée aux travailleurs du secteur alimentaire.

RECOMMANDATIONS EN MATIERE DE RECHERCHE

- Les légumes et les fruits frais sont à considérer comme des aliments à risque élevé. Alors que les voies de transmission des virus dans ces aliments sont mal connues, on admet en général que l'eau constitue une voie de transmission potentielle. En Belgique, nous disposons de peu de données sur la présence de virus dans les différents types d'eau utilisés dans la production primaire de plantes. Il est nécessaire de rechercher le lien entre la contamination virale et la présence d'indicateurs fécaux et de pathogènes bactériens.
- De plus, les facteurs de risque de contamination découlant du contact entre les eaux d'irrigation polluées et les légumes et fruits frais sont mal connus. Par ailleurs, on ne dispose guère d'informations sur la capacité des virus d'origine alimentaire à s'attacher, à adhérer et/ou à pénétrer dans les tissus des fruits et légumes ainsi que sur leur survie dans la niche écologique que sont les cultures agricoles.
- Davantage de données sur la stabilité des virus d'origine alimentaire soumis aux technologies de transformation alimentaire sont nécessaires. On a émis l'hypothèse que les virus sont plus stables dans cet environnement que les bactéries. Ce point requiert un examen plus approfondi. Différents virus modèles devraient être utilisés afin de déterminer les taux d'inactivation.
- Il convient d'examiner la résistance des virus sous différentes conditions physiques et chimiques imitant celles qui sont utilisées durant les processus de production.
- Il faut évaluer l'efficacité des biocides désinfectants contre les virus d'origine alimentaire typiques.
- La présence de virus d'origine alimentaire ou de virus apparentés chez les animaux domestiques exige une meilleure compréhension de leur transmission zoonotique éventuelle.
- Des données sur l'épidémiologie moléculaire des norovirus humains et animaux et des HEV (risque zoonotique et réservoir animal) sont nécessaires pour l'intervention future et le programme de prévention basés sur leur rôle d'agent zoonotique potentiel ou sur la présence d'un réservoir animal.
- Il faut envisager des études prospectives portant sur l'interaction virus-hôte pour les virus susceptibles d'émerger, tels que les HEV ou les virus Aichi.

- Il convient de mettre au point des méthodologies nouvelles capables de distinguer les virus d'origine alimentaire infectieux des virus non infectieux. Une meilleure évaluation des risques requiert la quantification de ces virus ainsi qu'une meilleure appréciation des doses infectieuses pour l'être humain. Cela contribuera à déterminer le risque en termes de santé publique si des virus sont détectés dans les denrées alimentaires, l'eau ou l'environnement à l'aide de techniques moléculaires telles que la (RT)-PCR.

ANNEXE

VIRUSES AND FOOD: SCIENTIFIC REPORT

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Ce groupe rédactionnel a été présidé par **Etienne THIRY**.

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ABBREVIATIONS

AFSSA	Agence Française pour la Sécurité Sanitaire des Aliments
ALT	Alanine transaminase
CaCV	Canine calicivirus
CDC	Centres for disease control and prevention
EFSA	European Food Safety Authority
ELISA	Enzyme Linked ImmunoSorbent Assay
FAO	Food and Agriculture Organization of the United Nations
FBVE	Foodborne Viruses in Europe network
FCV	Feline calicivirus
FDA	Food and drug administration
F+RNA	Name of a (coli)phage
GAP	Good Agricultural Practices
GHP	Good hygiene practices
GMP	Good manufacturing practices
HAV	Hepatitis A virus
HBGAs	Histo-Blood Group Antigens
HEV	Hepatitis E virus
HHP	High hydrostatic pressure
HIV	Human immunodeficiency virus
HS	Hepatitis-splenomegaly syndrome (HS syndrome)
Ig	Immunoglobulin
MuNV	Murine norovirus
MS2	Name of a enterobacteriophage
NoV	Norovirus
ORF	Open reading frames
PAA	Peroxyacetic acid
PBS	Phosphate buffered saline
PEC	Porcine enteric calicivirus
PEG	Polyethylene glycol
RNA	Ribonucleic acid
RT	Room temperature
RTE	Ready to eat food
RT-PCR	Reverse Transcriptase - Polymerase chain reaction
SARS	Severe acute respiratory syndrome
SaV	Sapovirus
TBEV	Tick borne encephalitis virus
US-USA	United States of America
UV	Ultraviolet
WHO	World Health Organisation

INTRODUCTION

Foodborne viruses are currently recognized as a major public health issue in many parts of the world. The purpose of this report is to review these viruses, which pose a threat to humans who consume contaminated food, and to issue recommendations aimed at improving the control of foodborne viral infections.

Foodborne viruses are pathogenic viruses found in food. They are transmitted to humans who ingest a contaminated food item. The main foodborne viruses infect and replicate only in humans. Their chief transmission route is usually person-to-person contact. As these viruses are relatively stable in the environment and may survive different food production processes, infected food-handlers can contaminate all kinds of food products. The food is merely a vector for onward transmission. This type of transmission can be defined as food-handler transmission.

Other viruses infect and replicate in animals. Some of them may also have the potential to infect humans. These so-called zoonotic viruses can infect humans through direct contact with the animal or its excreta. This is called zoonotic transmission. Infection can also be the outcome of consuming meat and meat products from infected animals. This is known as direct zoonotic foodborne transmission. Conversely, indirect zoonotic foodborne transmission occurs if infected animals shed the virus in their excreta, which results in a superficial contamination of meat, meat products or other kinds of food products.

Zoonotic foodborne transmission and food-handler transmission are very different routes of transmission. Managing them successfully therefore requires taking into account the different characteristics of the viruses.

Foodborne⁽¹⁾ and waterborne viral infections are increasingly recognized as causes of illness in humans. This is partly due to changes in food processing, consumption patterns, and the globalisation of the food trade, but also to our increased awareness of viral infections and the improved detection methods that advances in molecular techniques have led to.

Only a few countries actively look for viruses in foodborne outbreaks¹. According to the 2006 EFSA report, foodborne outbreaks caused by viruses are only notified in 8 countries (EFSA, 2006). The Centres for Disease Control and Prevention (CDC) report more foodborne outbreaks caused by viruses in the United States of America (USA) than they do in Europe: thus, 1.7% of these outbreaks were attributed to viruses in Europe in 2006, whereas they totalled 33% in the USA between 1998 and 2002 (Lynch *et al.*, 2006). Foodborne outbreaks caused by viruses are clearly being underreported. There are probably several explanations for this, e.g. the fact that those involved may not be aware of the danger or that these viruses go undetected because they are not being searched for.



¹ A foodborne outbreak is defined by the Zoonoses Directive 2003/99/EC as "an incidence, observed under given circumstances, of two or more human cases of the same disease and/or infection in which the observed number of cases exceeds the expected number and where the cases are linked, or are probably linked, to the same food source".

The most important viruses in terms of the number of cases involved and the severity of the illness are noroviruses and the hepatitis A virus. Other important foodborne viruses are the hepatitis E virus (HEV), human rotaviruses and human sapoviruses. Most of these viruses have a faecal-oral route of transmission. Other viruses are of minor importance: astroviruses, adenoviruses, aichivirus, enteroviruses and tick-borne encephalitis virus (TBEV). Some of these viruses are not detected on a routine basis and this may also contribute to the underreporting of cases. Table 1 lists the foodborne viruses with some of their characteristics and their priority level.

Moreover, there appears to be a broad host spectrum. It can also be hypothesized that there is zoonotic transmission of viruses like HEV, which was identified in pigs, and rotavirus, which can cross the species barrier but for which human beings are the main reservoir. There is no evidence yet for the zoonotic transmission of noroviruses, although the latter have not only been detected in humans, but also in several animal species, e.g. cattle, swine, sheep, mice, dogs and lions.

An exhaustive study would go beyond the scope of this report. The decision was therefore made to focus on viruses with intestinal excretion, for which there was shown to be a risk of transmission to humans by food or water ingestion. As a result, this report will deal primarily with the viruses below, which are listed according to their potential impact on human health:

Level 1: noroviruses and hepatitis A virus;

Level 2: hepatitis E virus, rotaviruses and sapoviruses;

Level 3: aichi virus, tick-borne encephalitis virus, louping ill virus, astroviruses, adenoviruses types 40 and 41, and enteroviruses.

In this context, influenza virus (detection in faeces of children with influenza A; highly pathogenic avian influenza H5N1), rabies virus, hantavirus, Nipah virus and severe acute respiratory syndrome (SARS) coronavirus were excluded because their significance as foodborne viruses is currently considered negligible.

TABLE 1. **List of foodborne viruses**

The priority given to these viruses stems from the outcome of a survey carried out among the experts of the working group.

Virus	Classification	Genome	Foodborne transmission	Food-handler transmission	Zoonotic transmission	Clinical signs	Reported foodborne outbreaks	Priority level
Hepatitis A virus (HAV)	<i>Picornaviridae</i> Hepatovirus	ssRNA positive	Proven	Proven	Not documented	- Hepatitis - Never chronic - 4 weeks incubation - Usually asymptomatic under 6 years of age	yes	1
Noroviruses	<i>Caliciviridae</i> Norovirus	ssRNA positive	Proven	Proven	Not documented	- gastroenteritis - 1-2 days incubation	Yes	1
Sapoviruses	<i>Caliciviridae</i> Sapovirus	ssRNA positive	Proven	Proven	Not documented	- gastroenteritis - 1-2 days incubation	Yes	2
Hepatitis E Virus	<i>Virus family unassigned</i> (proposed <i>Hepeviridae</i>)	ssRNA positive	Proven	Possible	Proven	- Hepatitis - only chronic in immuno-suppressed patients - 4 weeks incubation - Fulminant hepatitis (1% to 30% of pregnant women)	Yes	2
Rotaviruses	<i>Reoviridae</i> Rotavirus	dsRNA segmented	Proven	Suspected	Proven	- gastroenteritis - asymptomatic in adults - 3 days incubation	No	2
Aichi virus	<i>Picornaviridae</i> Kobuvirus	ssRNA positive	Proven	Not documented	Not documented	- gastroenteritis	Yes	3
Tick-borne encephalitis virus (TBEV) and louping ill virus	<i>Flaviviridae</i> Flavivirus	ssRNA positive enveloped	Proven	Not documented	Proven	- fever, neurologic symptoms - 7-14 days incubation	Yes	3

Virus	Classification	Genome	Foodborne transmission	Food-handler transmission	Zoonotic transmission	Clinical signs	Reported foodborne outbreaks	Priority level
Astrovirus	<i>Astroviridae</i>	ssRNA positive	Proven	Possible	Not documented	- gastroenteritis - 1-4 days incubation	No	3
Adenovirus types 40 and 41	<i>Adenoviridae</i> <i>Mastadenovirus</i>	dsDNA	Possible	Possible	Not documented	- gastroenteritis - 3-10 days incubation	No	3
Enteroviruses	<i>Picornaviridae</i> <i>Enterovirus</i>	ssRNA positive	Possible	Possible	Not possible (human virus)	- asymptomatic, fever, neurological symptoms, uncommon gastroenteritis	No	3
Influenza A virus	<i>Orthomyxoviridae</i> <i>Influenza A</i>	ssRNA negative segmented enveloped	Possible	Not documented	Proven	- flu symptoms - systemic avian influenza	No	4
Rabies	<i>Rhabdoviridae</i> <i>Lyssavirus</i>	ssRNA negative enveloped	Unlikely	Unlikely	Proven	- encephalitis	No	4
Hantavirus	<i>Bunyaviridae</i> <i>Hantavirus</i>	ssRNA negative segmented enveloped	Unlikely	Unlikely	Proven	- flu-like symptoms - renal syndrome - haemorrhagic fever	No	4
Nipah virus	<i>Paramyxoviridae</i> <i>Henipavirus</i>	ssRNA negative enveloped	Possible	Not document	Proven	- subclinical infection - flu-like symptoms - encephalitis	No	4
SARS	<i>Coronaviridae</i>	ssRNA negative enveloped	Possible	Not document	Proven	- fever - respiratory symptoms - pneumonia	No	4

ss: single-stranded; ds: double stranded

Chapter I - HUMAN INFECTIONS AND DISEASES CAUSED BY FOODBORNE VIRUSES

Noroviruses

Noroviruses (NoVs) are small non-enveloped viruses with a positive-sense, single-stranded RNA genome. The genus *Norovirus* belongs to the *Caliciviridae* family and is divided into 5 genogroups (GI to GV). As NoVs have a great genetic diversity, genogroups are subdivided into genetic clusters, called genotypes. As regards genogroups I and II, which are the most important for humans, there have been 8 and 17 genotypes described, respectively (Zheng *et al.*, 2006).

Hosts

Humans

Only GI, GII and GIV strains infect humans. NoVs are not only the main cause of epidemic nonbacterial gastroenteritis worldwide, but they are also a common cause of sporadic cases of gastroenteritis.

Age-related susceptibility. All age groups can be infected by NoVs. NoV infections cause notable problems in several special populations. The elderly, immunocompromised patients, infants, and others with serious underlying medical conditions may be more severely affected.

Immune response. Immunity is strain specific and re-infection can be induced following challenge with a strain that is serologically distinct from the one responsible for the previous infection. With the immunity that results from infection usually being short-lived, NoVs can reinfect previously infected hosts (Matsui *et al.*, 2000). The immune response is barely understood. Acquired immunity may protect on a mucosal level (Lindesmith *et al.*, 2005). Cell-mediated immune response studies have shown a preferential but not exclusive Th1 cytokine response following NoV challenge (Lindesmith *et al.*, 2005).

Genetic resistance of hosts. Some patients are genetically resistant to NoV infection due to genetic factors such as ABO blood type and secretor status (Kindberg *et al.*, 2007).

Animals

NoV has been described in several animal species. The Jena and Newbury 2 genotypes, in genogroup III, have been detected in non diarrheic and diarrheic faeces of calves and bovines (Mauroy *et al.*, 2008b; 2009a). Murine NoVs (MuNV) have been isolated from laboratory mice. The prevalence of MuNV is high in laboratory facilities and the infection is asymptomatic in wild type laboratory mice. Other animal strains that are genetically closer to human strains have been detected. Porcine NoVs have been detected in faecal samples of asymptomatic adult swine and were classified into genogroup II (Wang *et al.*, 2005; Mauroy *et al.*, 2008a). A strain that clustered into genogroup IV was isolated from hemorrhagic diarrhoea from a lion cub (Martella *et al.*, 2007) and recently from a dog (Martella *et al.*, 2008).

NoVs have a wide range of hosts but until now no zoonotic transmission has been described despite the relative closeness between some animal and human strains (Scipioni *et al.*, 2008b).

Pathogenesis

With no efficient cell culture system or convenient animal model available for human NoVs, the conclusions regarding their pathogenesis were drawn on the basis of studies in which the disease was experimentally induced in human volunteers.

Exposure and dose-response analysis

It is commonly accepted that the infectious dose is very low, but few studies could quantify it. Expert opinion placed the infectious dose at less than 10 to 100 virions (Atmar *et al.*, 2006).

Entry

NoV enters the human body mainly by the oral route (infection by inhaling aerosols produced by explosive vomiting has been described). Virions are acid stable, allowing them to resist the low pH levels in the stomach.

Dissemination in the body

NoVs are assumed to replicate in the upper intestinal tract, where they cause histopathological lesions.

Organ infection

Target cells, organs and tissue: there is limited information available. Lesions were shown to have occurred at the duodeno-jejunal junction.

Excretion

NV is excreted in faeces and vomit and can be shed in stool for several weeks after recovery from illness (Rockx *et al.*, 2002).

Transmission

NoV is mainly transmitted by the oral-faecal route, often through the ingestion of a faecally contaminated vehicle (food or water). Food can be contaminated at its source through the environment or after having been handled by infected individuals. Person-to-person transmission can be very significant, especially in community facilities, which are responsible for the high rate of secondary attacks in NoV outbreaks. Transmission through contact with contaminated surfaces makes it difficult to control the outbreaks.

Carriage

Up to one third of those infected during experimental challenge studies show subclinical infection. Prolonged infection has been described in immunocompromised patients shedding NoVs for periods of time ranging from several months up to several years (Carlsson *et al.*, 2009).

Passive carriage

Human. Virions are resistant in the environment and can be carried on hands and clothes.

Animals. Shellfish (bivalve molluscs) are able to concentrate virions in their digestive gland and are therefore a common source of foodborne outbreaks if eaten raw or insufficiently cooked. No animal NoV has been isolated from human stools but replication of a human GII NoV was demonstrated in gnotobiotic pigs (Cheetham *et al.*, 2006). Moreover, sequences closely related to GII.4 human NoV strains have been detected in swine and cattle (Mattison *et al.*, 2007b).

Clinical signs and pathology**In humans**

Symptoms appear 12 to 72 hours after ingestion: they may be gradual or abrupt. Vomiting and/or diarrhoea are the main signs but their predominance varies from person to person. Nausea, abdominal pain, abdominal cramps, anorexia, headache, malaise, and low-grade fever also occur (Atmar *et al.*, 2006). The infection is generally mild and self-limiting, patients usually recover within 3 days. Lesions have been found at the duodeno-jejunal junction in symptomatic as well as asymptomatic patients. Intestinal villi appear blunted but the mucosa remains intact.

In animals

Few animal NoVs cause severe clinical signs; they are mostly benign enteric pathogens. Only MuNV causes severe histopathological changes and has a fatal outcome in immunocompromised mice with clinical signs of encephalitis, vasculitis in cerebral vessels, pneumonia and hepatitis (Scipioni *et al.*, 2008b).

Hepatitis A virus

Hepatitis A virus (HAV) belongs to the family *Picornaviridae*, genus *Hepatovirus*. It measures 27 nm and is non-enveloped. The virus contains four capsid proteins encompassing a positive-sense single-stranded RNA genome.

Hosts**Humans**

Age-related susceptibility. In most cases infected children under the age of 6 with HAV do not show any symptoms (<10% have jaundice) (Fiore, 2004). Among young adults, clinical manifestations occur in 76 to 97% of cases (from mild, an icteric illness to fulminant hepatitis) and 40 to 70% have jaundice (Baert *et al.*, 2007).

Immune response. Only one serotype of HAV has been found. Lifelong immunity follows HAV infection and probably vaccination. In natural infection, IgM increase rapidly and levels readily decline (after 6 months, 75% of patients are negative). IgG begins to rise early in the course of infection and remains detectable for life (Koopmans *et al.*, 2002).

Animals

There is only one HAV serotype and primates are the only natural animal host (Fiore, 2004).

Pathogenesis**Exposure and dose-response analysis**

Direct contact with a person infected with HAV or ingestion of contaminated food. The infectious dose is not known, but it is probably low (estimated between 10-100 viral particles) (AFSSA, 2007).

Entry

The usual route of infection is the ingestion of HAV.

Dissemination in the body

Once ingested, the HAV particles enter the body through the gastrointestinal tract. They then replicate in the liver. HAV is excreted in the bile. Finally, viruses are found in high concentrations in stool specimens (Baert *et al.*, 2007).

Organ infection

The primary site for replication is the hepatocyte (Hollinger *et al.*, 2007). The target organ is the liver (hepatocytes). A viremia is observed during the incubation period. It ends shortly after hepatitis develops (Hollinger *et al.*, 2007).

Excretion

Sites. HAV is excreted in the bile and in faeces. HAV was found in saliva and tonsils (Cohen *et al.*, 1989).

Duration. Infectivity peaks during the 2-week period before the onset of symptoms and decreases the week after (Fiore, 2004). Excretion begins 2 weeks before the onset of symptoms and shedding in the faeces can be detected for several weeks with sensitive techniques (Fleet *et al.*, 2000; Hollinger *et al.*, 2007).

Transmission

HAV is transmitted among humans via the faecal-oral route (direct contact with a person infected with HAV or ingestion of contaminated food) and is the most serious form of viral illness contracted through food (Jean *et al.*, 2001).

It could also occur after exposure to contaminated blood or blood products, but not to saliva and urine (Fiore, 2004).

Carriage

Subclinical infection. Patients with unapparent or subclinical infection have neither symptoms nor jaundice.

Passive carriage. Outbreaks among humans are not easy to report. This is especially true for the source and transmission routes of HAV because of the long incubation period (time from exposure to the onset of symptoms), which is 28 days (range: 15 – 50 days) (<http://www.cdc.gov/hepatitis/HAV.htm>). Animals do not play a role in the epidemiology of this virus and its disease.

Clinical signs and pathology

In humans

The symptoms start with fever, anorexia, nausea, vomiting, diarrhoea, myalgia and malaise. Jaundice, dark coloured urine or light coloured stools might be present at the onset or might develop/occur within a few days. For most people infected with HAV, the illness lasts for several weeks. The mortality rate is about 0.3% of reported cases. Among the over 50s, the mortality rate rises to 1.8%. HAV-infection causes a potentially severe but controllable loss of liver function and general malaise. Proper medical care will generally result in a full recovery of liver function and full clearance of the virus from the host, with effective and lifelong immunity against reinfection (Baert *et al.*, 2007; Fiore, 2004).

In animals

Non-human primates have been identified as a potential source of exposure to HAV. This usually concerns chimpanzees, which have infected caretakers and other zoo personnel in close contact with them (Hollinger *et al.*, 2007). Wild or captive monkeys can be infected with HAV but human beings seem to have a low susceptibility to simian strains (AFSSA, 2007).

Hepatitis E virus

The hepatitis E virus (HEV) is the only member of the genus *Hepevirus* in an unassigned virus family that is provisionally named *Hepeviridae*. This virus is the major cause of several outbreaks of waterborne hepatitis in tropical and subtropical countries and of sporadic cases of viral hepatitis in industrialized countries. The genome is single-stranded, linear with positive-sense RNA of about 7.2 kb in length. Four genotypes are distinguished. Viruses consist of only one serotype and are transmitted mainly by the faecal-oral route. The overall death rate among young adults and pregnant women in endemic countries ranges from 0.5 to 3% and 15 to 20%, respectively.

Hosts

Humans

Age-related susceptibility. For reasons that are still unclear, pregnant women, especially those in their third trimester, have a poor prognosis when infected with HEV. High rates of both infant and maternal mortalities have been widely reported. Most studies of HEV have documented acute infection primarily in older teenagers and adults between the ages of 20 and 50. However, instances of sporadic hepatitis have been reported in children aged from 2 months to 15 years. In non-endemic areas, a higher adult seroprevalence is observed in combination with a lower paediatric seroprevalence (Labrique *et al.*, 1999).

Immune response. Anti-HEV IgM are detected in experimental infections in macaques approximately 3 to 4 weeks after the infection and continue to be detectable for up to 3 months. This seems to be consistent with reports in humans. Anti-HEV IgG follow shortly after the detection of IgM. However, anti-HEV IgG peak several weeks later and can be detected many months and years after the infection (Goens and Perdue, 2004).

Animals

Many animal species, such as swine, birds and deer, are infected with an antigenically similar virus. A swine virus is the best candidate for causing a zoonotic form of HEV and seems to be cross-infective. Deer and avian strains have also been detected recently (Goens and Perdue, 2004). Other animals have been shown to be susceptible to infection with HEV and could serve as reservoir in nature. These animals are boars, camels, deer, horses, dogs, cats, mongooses, primates, cows, sheep, goats, chickens, rodents and water buffaloes (Mushahwar, 2008).

Pathogenesis

Exposure and dose-response analysis

The incubation period in human volunteers after oral exposure is four to five weeks but the route and the mechanism by which the virus reaches the liver from the intestinal tract remains unknown (Aggarwal and Krawczynski, 2000). The infectious dose has been determined on the basis of intravenous infection in primates: 100 infectious particles are sufficient to induce a productive infection.

Entry, dissemination in the body and organ infection

In humans, HEV can be detected in stools from approximately 1 week before the onset of illness and persist for as long as 2 weeks. HEV-RNA can then be detected for approximately 2 weeks in the faeces of most patients with acute hepatitis E by RT-PCR. In some cases, RT-PCR has yielded positive results for as long as 52 days after the onset of illness. The HEV-RNA has regularly been found in serum from practically all patients by RT-PCR during the first 2 weeks following the onset of illness. Prolonged periods of HEV-RNA positivity in serum ranging from 4 to 16 weeks have also been reported (Aggarwal and Krawczynski, 2000).

Experimental infection with HEV leads to varying levels of virus excretion. Liver enzyme elevations and histopathological changes in the liver have been demonstrated in several non-human primates. The average incubation period for acute hepatitis E is approximately 21 days. HEV-RNA, as detected by RT-PCR, appears in serum, bile and faeces a few days before the onset of the transaminase (ALT) rise. HEV may be released from hepatocytes into bile before the morphological changes in the liver peak, during the highly replicative initial phase of infection. The liver injury may be largely immune-mediated, especially as infiltrating lymphocytes in the liver have been found to have a cytotoxic/suppressor immunophenotype. It is not known why the liver damage is particularly severe in pregnant women infected with hepatitis E (Aggarwal and Krawczynski, 2000).

Excretion sites and duration

Viremia is thought to last between 14 and 28 days in most patients with clinical disease, although it may be prolonged in some patients. Viral shedding in stool has been shown to begin up to 9 days prior to the icteric phase of disease. Normally, faecal shedding lasts up to 14 days after the onset of illness, but there are reported cases in which it continued until the seventh week of illness (Labrique *et al.*, 1999).

Transmission

HEV is mainly an enterically transmitted pathogen that causes sporadic cases of acute hepatitis in industrialised countries and waterborne outbreaks in developing countries. There are four documented routes of transmission of HEV: drinking contaminated water (waterborne transmission); consuming raw or undercooked meat from infected wild animals such as boars and deer and domestic animals like pigs (zoonotic foodborne transmission); parenteral (bloodborne) transmission; and vertical transmission from mother-to-child (perinatal transmission) (Mushahwar, 2008).

Carriage

Subclinical infection. Some infected individuals have a milder clinical course and develop only non-specific symptoms that resemble those of an acute viral febrile illness without jaundice (anicteric hepatitis). In these patients, liver involvement is recognized only if laboratory analyses are performed. In its most benign form, HEV infection is entirely unapparent and asymptomatic and passes unnoticed. The exact frequencies of asymptomatic infection and of anicteric hepatitis are not known but probably far exceed that of icteric disease as, in disease-endemic areas, a large proportion of individuals who test positive for anti-HEV antibodies do not recall having had jaundice (Aggarwal and Krawczynski, 2000).

Chronic infection. The illness is usually self-limiting and typically lasts 1 to 4 weeks. There is no evidence of chronic hepatitis or cirrhosis following acute hepatitis E. A few patients, however, have a prolonged clinical illness with marked cholestasis (cholestatic hepatitis), including persistent jaundice and prominent itching. The prognosis is good as jaundice finally resolves spontaneously after 2 to 6 months (Aggarwal and Krawczynski, 2000). Chronic infection has been detected in organ transplant patients, with the virus found in blood and stool (Kamar *et al.*, 2008).

Clinical signs and pathology

In humans

Typical hepatitis E symptoms include jaundice, dark urine, anorexia, enlarged tender liver, elevated ALT levels and abdominal pain accompanied by nausea, vomiting and fever. The disease may range in severity from sub-clinical to fulminant during pregnancy, where the death rate approaches 15 – 20% in endemic countries. Common complications during pregnancy may include encephalopathy, disseminated intravascular coagulation, death of the mother and foetus, abortion, premature delivery, or death of a live-born baby soon after birth (Smith, 2001; Goens et Perdue, 2004; Mushahwar, 2008). Chronic disease may develop in organ transplant patients (Kamar *et al.*, 2008).

In animals

HEV infection does not cause clinical illness in swine. Experimental exposure of 2 to 4 week old swine to human and swine HEV showed no evidence of clinical disease or elevation of liver enzymes (Halbur 2001; Williams 2001). All infected swine developed anti-HEV antibodies; most within 27 days post-inoculation. Experimentally inoculated swine did develop mild hepatic lesions consisting of

enlarged hepatic and mesenteric lymph nodes, multifocal lymphoplasmacytic hepatitis and hepatocellular necrosis. Swine infected with the human HEV strain had more severe lesions than swine infected with the U.S. swine HEV strain (Halbur 2001). Experimental infection of pregnant gilts with HEV showed no evidence of clinical disease in the gilts or piglets (Kasorndorkbua 2003).

Most chickens are subclinically infected; this is similar to the situation in swine. The HS (hepatitis-splenomegaly) syndrome in US chickens is characterized by increased mortality in birds (primarily broiler breeder) from 30 to 72 weeks of age, the presence of an enlarged liver and spleen, regressive ovaries and red fluid in the abdomen, whereas young birds are more often asymptomatic or subclinical. In an experimental infection, seroconversion occurred between 12 and 21 weeks in healthy chickens. Lesions in the liver ranged from multifocal patches to extensive necrosis and haemorrhages (Goens and Perdue, 2004).

Rotaviruses

Rotaviruses are non-enveloped viruses with a double capsid, giving them a wheel-like appearance in electron microscopy. The genome consists of 11 segments of double stranded RNA. RNA segments reassort frequently during dual infections if the viruses are from the same rotavirus group (Estes and Kapikian, 2007). The genus *Rotavirus* belongs to the family *Reoviridae*. Seven groups need to be distinguished but only A, B and C rotavirus groups are found in both humans and animals. Humans are mainly affected by group A viruses.

Hosts

Humans

Usually, children under the age of 3 are susceptible to group A rotavirus infections. Group B rotaviruses primarily cause epidemics of severe diarrhoea in adults in China. Group C rotaviruses have been sporadically reported in faecal specimens from children with diarrhoea in Japan (Svensson, 2000). The high prevalence of rotavirus antibodies in adults indicates that subclinical reinfections are common. Cell mediated immunity is important in limiting and clearing virus infection. Antibodies in the human small intestine were the primary determinant of resistance to rotavirus illness. Though breastfeeding does transfer immunity to newborn and young infants, there only appears to be a modest lasting protective effect (Estes and Kapikian, 2007).

Animals

Group A rotaviruses are the most prevalent and are associated with diarrhoea in calves. A low virulent strain can cause diarrhoea in a 2-day old newborn calf. A virulent strain is characterized by its pathogenic ability to cause diarrhoea in a 6-week old calf (Thiry, 2007). Passive immunization of the calf by correctly administering colostrum could protect the animal. This argues in favour of vaccinating cows during gestation (Thiry, 2007).

Pathogenesis

The transmission occurs by the faecal-oral route. The duration of rotavirus shedding is 4 to 29 days with a median of 7 days. The target cells of rotaviruses are differentiated enterocytes in the small intestine, near the tips of the villi (Estes and Kapikian, 2007). The infective dose in human beings is probably 10 – 100 infectious viral particles (Baert *et al.* 2007). A person with rotavirus diarrhoea often excretes large numbers of viruses: 10^8 – 10^{10} infectious particles/ml of faeces. The same quantity of infectious particles is excreted by infected calves.

Rotaviruses are resistant to physical inactivation. Calf rotaviruses in faeces remain infectious for 7 months when kept at room temperature.

Respiratory symptoms occur in a proportion of patients with rotavirus gastroenteritis, but the respiratory route is not the usual mode of transmission (Cook *et al.*, 2004). Rotavirus infections display a seasonal pattern of infection in developed countries, with epidemic peaks occurring during the winter season (Fleet *et al.*, 2000).

Adults (of all species) appear to undergo rotavirus reinfection commonly but characteristically with minimal or no clinical manifestation.

Rotavirus can cross the species barrier and infect other animal species. Human rotavirus is transmissible to cattle but causes less severe diarrhoea than the disease caused by the homologous virus. Similarly, many human infections with bovine rotavirus are asymptomatic (Cook *et al.*, 2004). Furthermore, reassortant bovine x human viruses have been isolated. Wild reassortants between porcine and human rotavirus were observed in Brazil (Santos *et al.*, 1999).

Several case studies have indicated that humans can be infected through direct contact with household pets like dogs and cats. In addition, household contamination of objects and surfaces with faeces from infected animals may also result in the transmission of rotaviruses to humans (Cook *et al.*, 2004).

Rotaviruses have also been shown to be present in bivalve shellfish grown in contaminated waters. However, rotaviruses have not been linked to infectious disease following seafood consumption (Lees, 2000).

Waterborne outbreaks of group B rotaviruses were reported in China. In Japan, a large foodborne outbreak affecting schoolchildren was reported for group C (Svensson, 2000).

Clinical signs and pathology

In humans

The illness manifests itself after a 1-2 day-incubation and is characterized by the sudden onset of acute, watery diarrhoea and vomiting, often accompanied by fever in young children under 3 years of age. In this age group it is the first cause of diarrhoea worldwide. It results in a high burden of disease in all countries and is an important cause of death through dehydration in developing countries. Symptoms may persist for several days or longer, leading to dehydration (Estes and Kapikian, 2007; Fleet *et al.*, 2000).

Chronic symptomatic infection may occur in immunodeficient children. Viruses can be detected in the liver and kidneys. Rotaviruses do not appear to play an important role in diarrhoea in adults infected with the human immunodeficiency virus (HIV) (Estes and Kapikian, 2007).

Wild-type rotavirus infection in children may induce invagination through intestinal lymph node thickening (Estes and Kapikian, 2007). Group B rotaviruses cause epidemics of severe diarrhoea in adults.

In animals

In calves, the incubation period could be extremely short (12 hours after infection), the clinical signs include lethargy, anorexia and diarrhoea. The virus can infect adult animals and be excreted in faeces, but the clinical signs are non-existent (Thiry, 2000). Rotavirus infections in dogs are commonly subclinical (Cook *et al.*, 2004).

Sapoviruses

Sapoviruses (SaV) are positive, single stranded RNA viruses with a poly-A tail. They belong to the *Caliciviridae* family, genus *Sapovirus*. Five genogroups have been described. They were discovered in 1977 as the causative agent of an outbreak of gastroenteritis in an infant home in Sapporo, Japan (Chiba *et al.*, 2000).

Hosts

Humans

SaV have been identified worldwide and human SaV strains are classified in genogroups I, II, IV and V.

Age-related susceptibility. Sapoviruses seem to play a more predominant role in infantile gastroenteritis than in foodborne outbreaks. These viruses are most common in children (under the age of one) and the elderly, especially in immunocompromised patients (Rodriguez-Guillen *et al.*, 2005).

Immune response. SaV are antigenically distinct from noroviruses (Cubitt *et al.*, 1987). Antibody titres rise rapidly after primary infection and are maintained for at least 3 months. Pre-existing antibodies result in protection against homologous infection (Nakata *et al.*, 1985). Virtually all 5-year old children have been infected with sapoviruses.

Animals

Genetically diverse SaV strains have been identified in pigs (Guo *et al.*, 1999, Mauroy *et al.*, 2008a). The prevalence among pigs was found to be 62% in three US states, with the highest prevalence found in postweaning pigs (Wang *et al.*, 2006). Korea also reports strong prevalences (Yu *et al.*, 2008). The majority of porcine strains genetically cluster in genogroup III, but newly identified strains could form another new cluster. As recombination occurs in human or porcine SaV (Katayama *et al.*, 2004; Wang *et al.*, 2005), pigs could be the source of recombination between human and porcine strains. In fact, intergenogroup recombination has already been described (Hansman *et al.*, 2005). However, to date, no human strains have been identified in pigs. Sapoviruses were found in diarrhoeic minks in the USA (Guo *et al.*, 2001a).

Pathogenesis

Very few volunteer studies have been carried out on human SaV. These viruses are difficult to grow on cell culture. The faecal-oral route is undoubtedly the main route of infection and it is assumed that replication occurs in the upper intestinal tract. The SaV receptors have not been described yet. They do not appear to be related to HBGAs, in contrast to NoV (Shirato-Horikoshi *et al.*, 2007).

The Porcine Enteric Calicivirus (PEC) Cowden strain, the only SaV strain known to replicate in cell culture, has been used to describe the pathogenesis and pathology of SaV infection. Mild to severe villous atrophy was shown to occur in the duodenum and the jejunum after oral inoculation. Electron microscopy analysis revealed moderate to severe villi shortening and blunting. Shedding in stool persists for at least 7 days. It peaks between days 4 and 7. One amino acid mutation in the capsid region and two in the polymerase region were noted in the PEC culture-adapted strain compared to the native strain. Gnotobiotic pigs infected with that strain show milder histopathological lesions. Antigens were identified in villous enterocytes of the proximal intestine, none were found in the colonocytes. Viremia was detected in orally infected pigs. Intravenously inoculated pigs develop diarrhoea and villous atrophy with no crypt enterocyte infection (Guo *et al.*, 2001b).

Clinical signs and pathology

In humans

An incubation period of 24-48 hrs is usually noted. Diarrhoea and vomiting are the main symptoms of clinical infection, yet there are also high rates of asymptomatic infections. Other symptoms too have been said to be associated with SaV infection (headaches, abdominal cramps, myalgia, nausea, hyperthermia). The symptoms are generally milder than those that have been reported in cases of norovirus-associated gastroenteritis (Moreno-Espinoza *et al.*, 2004).

In animals

Both clinical signs of gastroenteritis and asymptomatic infection have been reported to occur in pigs infected with SaV (Guo *et al.*, 2001b). A strong morbidity was reported in minks during a gastroenteritis outbreak on a farm. The kits in particular developed diarrhoea that persisted for several days (Guo *et al.*, 2001a).

Aichi virus

The Aichi virus belongs to the genus *Kobuvirus* of the *Picornaviridae* family. This virus was first identified in Japan in 1989 as the likely cause of oyster-associated gastroenteritis in a Japanese patient. It can be responsible for cases of gastroenteritis after eating shellfish. The presence of virus-specific antigen and of viral RNA was demonstrated in faecal specimens collected from Japanese gastroenteritis outbreaks; up to 32% of these were associated with the Aichi virus. The virus has also been isolated from Pakistani children and from Japanese tourists returning from Southeast Asian countries and suffering from gastrointestinal symptoms.

Further epidemiological studies revealed the presence of the virus in stool samples taken from patients suffering from diarrhoea in Brazil and Germany (Oh *et al.*, 2006). In France, the virus was identified in 10 out of 63 shellfish samples (Krol *et al.*, 2008). It was associated with a low incidence of gastroenteritis outbreaks in general but it could be involved in half of the oyster-associated outbreaks (Ambert-Balay *et al.*, 2008). In Japan and Germany, Aichi virus infection was highly seroprevalent (more than 70%) in the sampled population (Oh *et al.*, 2006). On the other hand, virus infection was not detected in the Netherlands as a cause of gastroenteritis (Svraka *et al.*, 2007). Kobuviruses were also identified in cattle from Asia and Europe (Yamashita *et al.*, 2003; Mauroy *et al.*, 2009b) and in pigs from China and Hungary (Reuter *et al.*, 2008).

These data suggest that this virus infection has a worldwide distribution, that it is present in shellfish, that it is probably transmitted to humans through shellfish and that it is associated with gastroenteritis outbreaks, with an incidence that varies greatly from one country to another.

Other potential foodborne viruses

Tick-borne encephalitis virus

The TBE virus belongs to the genus *Flavivirus* of the *Flaviviridae* family. It is the only known foodborne virus that is not transmissible through the faecal-oral route. It is also the only enveloped virus known to be associated with foodborne infections. Dairy animals are infected, mainly in central Europe, via tick bites. Infected animals shed the virus in their milk, which may in turn infect humans when it is ingested without having been pasteurized (Svensson, 2000). Recent cases of human TBEV infection following the ingestion of goat cheese were reported in Austria (ProMED, 2008). TBEV can also be transmitted through blood transfusion (Leiby *et al.*, 2004).

Louping ill virus is another tick-borne flavivirus whose natural hosts are sheep and grouse (*Lagopus lagopus*). It is a zoonotic virus that can infect a wide variety of mammals and birds, as dead-end hosts. It can be transmitted to humans through ewe milk and aerosol (see OIE report at http://www.cfsph.iastate.edu/Factsheets/pdfs/louping_ill.pdf)

Astroviruses

Astroviruses were first identified in 1975 in the stool of a child with diarrhoea. They are responsible for a few percentages of the cases of acute gastroenteritis. The epidemiological evidence of foodborne transmission is limited (Svensson, 2000).

Adenoviruses types 40 and 41

Only the adenovirus types 40 and 41 induce gastroenteritis, with most cases involving young children (Svensson, 2000). They were identified in food such as shellfish. Foodborne transmission is not documented.

Enteroviruses and parechoviruses

These viruses belong to the *Picornaviridae* family. The genus *Enterovirus* includes human polioviruses, echoviruses, enteroviruses 68-71 and coxsackieviruses. The genus *Parechovirus* includes two human parechoviruses formerly classified as echoviruses. Various pathologies are associated with enteroviruses, the most prominent being poliomyelitis and meningitis. Nowadays, polioviruses are not considered a problem in Europe. Poliovirus type 2 is eradicated but types 1 and 3 still circulate in parts of Nigeria, India and Pakistan. Eradication is under way but not yet complete.

Influenza A virus

Infectious avian influenza A virus has been cultured from frozen exported meat, raising questions about its potential dissemination in the food chain (Serena *et al.*, 2006). There is no evidence of the influenza A virus being transmitted to humans via pork and meat products.

Rabies virus

Contamination through the ingestion of a rabid carcass is very rare (Anonymous, 1985). It is highly unlikely that a rabid carcass should be consumed, making this an implausible route of transmission,

Hantavirus

The contamination of food by hantaviruses is a secondary way of infection. The probability of hantavirus contamination is very low, with this virus being transmitted by inhaling the urine of an infected rodent.

Nipah virus and severe acute respiratory syndrome (SARS) virus

The faecal-oral spread of these primarily respiratory pathogens has been proven under special conditions (FAO/WHO, 2008).

Chapter II - DIAGNOSTIC METHODS

Introduction

Because of the low infectious dose of human enteric viruses, food can only be considered safe from viral contamination if the viral load is below 10 to 100 particles. Consequently, the detection method for human enteric viruses in food products should be sensitive enough to detect such low doses. This means that the detection of human enteric viruses is more challenging in food products than in clinical samples, since infected individuals generally shed $>10^6$ virus particles per g stool. The methods used to detect bacterial pathogens in foods include enrichment steps and selective plating, which make it possible to grow the bacteria to detectable numbers and to suppress the accompanying bacterial flora. Such a strategy cannot be used to detect viruses, which can only replicate in cells or embryonated eggs. Furthermore, there are no *in vitro* cell lines available to grow NoV and wild type HAV strains do not grow well. Though several efforts have been made to cultivate NoV *in vitro* (Duizer *et al.*, 2004b), it has not been possible to find a routine cell culture system. Screening for low amounts of viral particles in food products uses molecular techniques such as reverse transcription-PCR (RT-PCR) and real-time RT-PCR (Scipioni *et al.*, 2008a; 2008c).

At present, there are no standard reference methods available to detect NoV and HAV in food products. With conventional RT-PCR assays, it is difficult to quantify the level of viral contamination. Real-time RT-PCR is a good alternative because of its high specificity, sensitivity and the possibility of quantification. Before applying molecular techniques, it is necessary to concentrate the virus particles to allow small volume PCR reactions. Furthermore, food substances should be eliminated as much as possible because these may inhibit the RT-PCR reaction. One drawback is that detecting viral nucleic acid with molecular techniques does not necessarily mean that there are infectious viral particles in the food product. However, the presence of viral nucleic acid does indicate that there has been contamination with human enteric viruses. This in turn means that there is a potential human health hazard.

Detection of viruses in food

Virus release from the food matrix, virus concentration and RNA extraction

Extraction methods are necessary to concentrate the viral material and to remove inhibitory components that are present in the food products. In the literature, two different approaches are described to concentrate viruses or viral nucleic acids. The first approach involves the straight extraction of total RNA (including viral RNA) from the sample and using it directly for RT-PCR detection. This method is recommended by the American Food and Drug Administration (FDA) (Goswami *et al.*, 2002).

The extraction of total RNA from food is often performed with the commercial phenol reagent TriZOL (Boxman *et al.*, 2006; Baert *et al.*, 2008d). In the second approach, virus particles are isolated from the food matrix prior to the extraction of the viral RNA. Here, virus concentration involves a series of consecutive steps during which a washing solution is added to elute the virus particles, followed by filtration, solvent extraction, primary polyethylene glycol (PEG) precipitation, secondary PEG precipitation followed by RNA extraction and RT-PCR (Legitt and Jaykus, 2000). Acid absorption–elution–concentration (Shieh *et al.*, 1999; Mullendore *et al.*, 2001), alkalic (LeGuyader *et al.*, 1994) or neutral (Atmar *et al.*, 1995) elution–concentration approaches have been described. PEG is generally used to precipitate the virus particles. However, various concentration steps have been suggested (Jaykus *et al.*, 1996; LeGuyader *et al.*, 1996; Lewis and Metcalf, 1988). The purification steps differ in terms of the kind of reagent that is used. This can be e.g. freon (Dix and Jaykus, 1998) or a mixture of chloroform and butanol (Atmar *et al.*, 1995). They also vary with respect to the number of purification steps and the sequence within the procedure in which the purification is performed.

Reverse-transcription-PCR

Viral RNA extracts are amplified and detected by RT-PCR. For real-time RT-PCR, intercalating dyes such as SYBR Green 1 as well as fluorescently labelled probes can be used. SYBR Green 1 binds to every double stranded nucleic acid that is generated during amplification. Consequently, this approach is less specific than fluorescently labelled probes (eg. TaqMAN) that bind a specific region of the amplified PCR product. There have been various real-time RT-PCR assays reported for the detection of NoV GI and GII. Most of these are TaqMAN-based methods, which target the ORF-1-ORF2 junction, i.e. the most conserved region of the NoV genome (Kageyama *et al.*, 2003; Hohne and Schreier 2004; Myrmel *et al.*, 2004; Pang *et al.*, 2004; Richards *et al.*, 2004; Schmid *et al.*, 2004; Gunson and Carman 2005; Jothikumar *et al.*, 2005; Loisy *et al.*, 2005). Several real-time RT-PCR assays have been described for HAV as well, most of which are TaqMAN-based and directed at the very well conserved 5' non-coding region of HAV (Abd el-Galil *et al.*, 2005; Jothikumar *et al.*, 2005; Costafreda *et al.*, 2006).

Detection of viruses in water

The enteric viruses described as foodborne viruses in this report are mainly transmitted by the faecal-oral route. These viruses are shed in human stool and end up in sewage. Sewage is normally treated and purified before it comes into contact with surface or seawater, possibly even drinking water. Whenever the water treatment is inadequate, there is a danger of it being (i) a direct source of infection in the case of contaminated drinking water or recreational water, or (ii) an indirect source of infection in the case of contaminated wash water or irrigation water for foods.

The volume of water that is needed for examination depends on the level of contamination and on the turbidity of the sample. Groundwater and drinking water will contain few viruses. Therefore, it will be necessary to process 100 L or more. As regards recreational or river waters, 10 L suffice. One litre is enough to analyse treated sewage, whereas in the case untreated sewage, 100 ml will do (Wyn-Jones *et al.*, 2001).

Viruses are small and cannot be concentrated by mechanical filtering (Fong *et al.*, 2005). The most widely applied concentration method is the adsorption–elution principle. Virus particle concentration is based on their natural or artificially manipulated charge. Most enteric viruses have a negative charge at ambient pH (Lipp *et al.*, 2001). Viruses that are negatively charged by nature can be trapped by the use of electropositive filters (Gilgen *et al.*, 1997; Haramato *et al.*, 2004; Katayama *et al.*, 2002). Electronegative filters can be used if the pH value of the water sample is lowered or if the virus particles are complexed with Mg^{2+} (Lodder *et al.*, 2005). Alternatively, borosilicate glass beads of 100-200 μm and glass wool evenly packed in a column at a density of 0.5 g cm^{-3} form good absorbents for viruses and can be used as a concentration method (Wyn-Jones *et al.*, 2001).

The viruses are eluted from the filters by means of a buffer that mostly includes beef extract (Gilgen *et al.*, 1997; Haramato *et al.*, 2004; Katayama *et al.*, 2002; Lodder *et al.*, 2005). Haramato *et al.* (2004) used NaOH instead of beef extract to elute viruses in order to avoid the potential inhibition of the molecular detection techniques. The elutes are further concentrated to 1-2 ml by centrifugal filtration (Centricon) or ultracentrifugation or are processed by means of a two-phase separation method (Poyry *et al.*, 1988). The latter uses polymers Dextran/PEG to separate virus particles during a particular phase (bottom- and interphase). The virus containing phase is purified by ultrafiltration or spin column gel chromatography with sephadex.

Viral RNA is isolated from the final volumes with commercially available RNA kits. It is detected with RT-PCR in a way that is similar to the protocols described for the detection in foods.

Indirect methods with indicators (human faecal contamination)

Foodborne viruses such as NoV or HAV are difficult to detect. As a result it has been suggested to look for human adenoviruses. The latter are frequently found in polluted water and identified in shellfish, yet they are rarely transmitted through food (Carter, 2005). They are detected by means of PCR. Human adenoviruses are reported to be more prevalent than enteroviruses and hepatitis A viruses (HAV) in different aquatic environments and are more prevalent than NoV in shellfish from different European countries (Municain-Mujika *et al.* 2000; Formiga-Cruz *et al.*, 2003). Nevertheless, Jiang *et al.* (2004) reported that HAV were detected in river water in California, in spite of the fact that there was no human adenovirus

found. With the relation to infectious units still unclear, this raises questions about the reliability of using human adenoviruses as an indicator.

Shellfish are subjected to regulations that are based on the use of traditional bacterial indicators of faecal contamination, such as faecal coliforms or *E. coli* in shellfish or shellfish growing waters (Lees, 2000). Depuration, which is used to reduce microbiological contaminants in shellfish, reduces the number of *E. coli* in oysters by 95%. However, only a 7% reduction of NoV was observed to have occurred after 48h (Schwab *et al.*, 1998). The increased resistance of viruses compared to indicator bacteria probably explains the fact that there is a low correlation between faecal coliform indicators and the presence of enteric viruses in shellfish and their harvesting water (Lees, 2000). Consequently, these hygiene indicators are not a reliable means to show that there is viral contamination.

Viral genome detection turned out to be better correlated for somatic coliphages than for coliforms in river water in France (Skraber *et al.*, 2004). Somatic and F-specific coliphages have a similar genomic structure (ss-RNA), in contrast to enteric viruses. They can be cultivated easily without high costs, which favours their role as indicators. There was also found to be a link between F+RNA phages and the presence of entero- and reoviruses (Havelaar *et al.*, 1993) in fresh water. F+RNA coliphages are divided in four main subgroups, with groups II and III closely linked to human faecal contamination and groups I and IV found in animal waste (Scott *et al.*, 2002). It has been suggested that coliphages are able to proliferate in the environment. This in turn casts doubt on their correlation with enteric viruses. Alternatively, phages from animal faeces can be differentiated from phages from human faeces by using specific *Bacteroides fragilis* strains, which are stable in the environment (Tartera and Jofre, 1987; Puig *et al.*, 1999). Gantzer *et al.* (1998) found that there is a close correlation between *B. fragilis* phages and enterovirus contamination. During a wastewater treatment failure, there was found to be an increase in both the number of enteroviruses and the concentration of *B. fragilis* phages, whereas somatic coliphages were a poor indicator for this fluctuating enterovirus concentration.

Besides shellfish and water quality, there have not yet been any data described on potential indicator micro-organisms for other food products. The proposed indicator organisms all have their own drawbacks and require further assessment in other environmental samples and foods.

Because in most cases, viral contamination results from contact with human faecal material, good agricultural practices (GAP) and good hygiene practices (GHP) are of major importance throughout the entire food chain.

Detection of viruses in human samples

Diagnosis of individual cases

Foodborne viral infection in humans can either be diagnosed directly, by detecting the virus or parts of it (norovirus, sapovirus, rotavirus), or indirectly, by identifying antibodies, particularly of the IgM class (hepatitis A, hepatitis E viruses). Some diagnostic techniques are widely available in clinical laboratories (hepatitis A IgM, rotavirus antigen detection), others are restricted to some laboratories (hepatitis E virus IgM, norovirus or sapovirus by RT-PCR). The current practical sensitivities and specificities of these tests are quite high.

Outbreak investigation

Diagnosing foodborne viral infections in humans can only contribute to the detection of foodborne outbreaks if an outbreak investigation is carried out. This in turn requires the public health inspector (*Médecin inspecteur d'hygiène / Arts infectieziekten*) to be informed. For diseases which currently have a low incidence, one or two cases are enough for an outbreak to be declared. Whilst this is of course out of the question for rotavirus infections, it is certainly possible for hepatitis E, hepatitis A or Sapovirus, as well as a group of norovirus infections.

Chapter III – THE IMPORTANCE OF FOODBORNE VIRUS OUTBREAKS

General

Viruses are the pathogens that are most commonly transmitted through food. In the United States, 66.6% of food related illnesses are caused by viruses, whereas for *Salmonella* and *Campylobacter*, these proportions reach 9.7% and 14.2%, respectively (Mead *et al.*, 1999). Viral gastroenteritis was reported to be the most common foodborne illness in Minnesota from 1984 to 1991. It was predominantly associated with the poor personal hygiene of infected food-handlers (Jaykus *et al.*, 1997). Noroviruses and HAV are currently recognized as the most important human foodborne pathogens in terms of the number of outbreaks and people affected in the Western World (Cliver, 1997). There have only been a few large rotavirus outbreaks caused by infected food, whereas waterborne HEV outbreaks have only occurred sporadically in Europe. (Koopmans and Duizer, 2004).

It is mandatory for European member states to report foodborne outbreaks to the EFSA. According to the 2006 EFSA Summary Report, eighteen member states and one non member state reported foodborne virus outbreaks. Foodborne viruses (adenovirus, norovirus, enterovirus, HAV and rotavirus) were responsible for 587 out of a total of 5 807 reported foodborne outbreaks, which means that they caused 10.2% of the outbreaks that were notified in 2006. Thus, there has been a marked increase in the number of viral foodborne outbreaks that are reported, compared to 2005 (5.8%). In previous years, *Salmonella* was the most common cause of foodborne outbreaks. Yet, in 2006, foodborne viruses became the second most frequent cause for the first time. With a total of 13 345 individuals concerned, foodborne viruses were found to be the second most important agent after *Salmonella* if the number of individuals infected was used as a criterion. However, the two illnesses turned out to differ greatly in terms of their severity. Thus, only 4% of the patients in foodborne virus outbreaks were admitted to hospital and 3 died, whereas in the case of foodborne *Salmonella* outbreaks, 14% of the patients were admitted to hospital and 23 died. Between 2005 and 2006, the number of outbreaks caused by viruses that were reported increased by 88% and the number of people affected almost doubled. It has been assumed that outbreaks caused by foodborne viruses were critically underreported in the past and that the data from 2006 probably reflect their true occurrence more accurately than those from previous years, when, in addition, fewer countries in general reported data on viruses.

When comparing data both across Europe and across the world, one needs to take into account the fact that not all countries have the required diagnostic capability and that the structure of the national surveillance systems differs greatly from one country to another (Lopman *et al.*, 2002a). Countries like UK and the Netherlands investigate outbreaks of gastroenteritis independently of their extent or possible mode of transmission. In Denmark and France, only the outbreaks that appear or are suspected to be foodborne from the onset are examined.

In Belgium, there is currently no specific procedure in place to trace all viral foodborne outbreaks and draw the link between human epidemics and food contamination. Since Belgium was reformed into a federal state with regions and communities, there has been a need for coordination between the different partners involved in outbreak monitoring. With food being a federal competency and person related matters such as illness belonging to the competencies of the Flemish, French and German communities, the data on foodborne outbreaks are scattered. The creation of a National Platform for Diseases Transmitted by Food in the Institute of Public Health has led to improved communication and information exchange and has enhanced the collecting of data from outbreak investigations and case-control studies. A field and laboratory scenario is currently being worked out in order to improve the linking of norovirus outbreaks to their foodborne cause and to shed light on the transmission routes of norovirus strains circulating in humans, animals and food. The molecular epidemiology of detected viruses will be of major interest to trace the outbreaks from their source to those infected, thus providing information on the circulation of the various norovirus strains, both from a geographical point of view and within the population.

Norovirus

Noroviruses are one of the primary causes of gastroenteritis in adults and often induce outbreaks. This viral pathogen is chiefly transmitted from person to person. However, foodborne transmission (by contaminated food and water or infected food-handlers) seems to be significant (Koopmans and Duizer, 2004). The part played by food or water in norovirus outbreaks was generally underestimated in the past. This was due to the lack of appropriate detection methods for noroviruses to confirm the presence of this etiological agent in food. With the recently improved norovirus-specific diagnostics (real time PCR methods), these viruses are being reported as the causative agents in outbreaks at an increased rate. In 2002, Lopman reported that only 5 out of 10 European countries had the required methodology to detect viruses in food. Today, there are more isolation and detection methods available for noroviruses in foods, but they are not easy to perform in a routine laboratory. Also, there is no official method yet (Rutjes *et al.*, 2006; Boxman *et al.*, 2006; Baert *et al.*, 2008c).

It is estimated that foodborne transmission accounts for 14% of norovirus infections. According to the data from 10 surveillance systems in the Foodborne Viruses in Europe network (FBVE), noroviruses were found to be responsible for more than 85% of all the cases of non bacterial gastroenteritis that were reported from 1995 to 2000. It is not always possible to determine whether the illness results from foodborne or person to person transmission. Foodborne transmission can occur in either of two ways: thus, the food items can be contaminated before they are harvested by washing or irrigation (like shellfish, soft fruits, vegetables) or during processing by a contaminated food-handler.

According to the 2006 EFSA report, more than 60% of all foodborne virus outbreaks were caused by caliciviruses, mostly noroviruses (i.e. 196 out of 315 outbreaks, representing 6 006 cases in 2005). They were the most common source of non-bacterial foodborne outbreaks. In 64% of the calicivirus outbreaks, the source of illness was unknown. The report also mentions that it is difficult to confirm the presence of noroviruses in food items because there is no internationally accepted protocol available for the moment. The most common known food vehicles were crustaceans and shellfish, mixed food and buffets and vegetables.

The location of exposure was reported in 83% of the calicivirus outbreaks, which totalled 295. The most common location of the exposure was private homes (23%), with an average of 7 people per outbreak concerned. In total, 45% of all patients were either infected in schools, kindergartens and residential institutions or in restaurants and cafés.

TABLE 2.
Reported norovirus outbreaks in Belgium
during the 2004-2007 period

Year	Number of reported norovirus outbreaks	Suspected food vehicle	Laboratory confirmed in food	Possible transmission route
2004	2	unknown	No	unknown
2005	1	pizza	No	unknown
2006	3	Composite meal, soup	Yes (2/3)	1/3 food-handler suspected
2007	10	Composite meal, soup, chicken with curry, meat stew, mashed potatoes, sandwiches	Yes (5/10)	8/10 food-handler or personnel serving the meal suspected

The causative agent remains unknown in 20 to 50% of the outbreaks that are reported in Belgium each year. Noroviruses are known to be an important cause of foodborne outbreaks and could be partially accountable for these cases. Besides the fact that an extraction and detection system for this virus has only been available for routine analyses in different kinds of foodstuffs since 2006, the actual number of norovirus infections is still being underestimated because of a low rate of reporting. This is due to the fact that the infection is normally self-limiting and there are no known complications. In many outbreaks, there were no patient samples analysed for noroviruses because these analyses are

not reimbursed in Belgium. A recent agreement between the Flemish Community and the National Reference Laboratory for foodborne outbreaks in Brussels makes it possible to analyse patient samples in suspected norovirus outbreaks. In some cases, there were no food samples analyzed because there were no leftovers.

In 2004, two general foodborne norovirus outbreaks were registered in Belgium. In total, 33 individuals became ill after eating at a restaurant, but the food source could not be traced down.

In 2005, there was one norovirus outbreak reported. 65 individuals became ill after dining in the restaurant (buffet) of a holiday park. The epidemiological investigation pointed to the pizza that was served as being the food that was most suspected to have caused it, but noroviruses could only be detected in the patients, not in the food.

In early 2006, a norovirus extraction and detection protocol was established in the laboratory for foodborne outbreaks. It used the extraction method procedure described by Baert *et al.* (2007).

In 2006, 3 NoV infection outbreaks were reported to the National Reference Centre for foodborne outbreaks (NRL-VTI). Two outbreaks occurred within the same institution, a care centre for the disabled. During the first episode, which happened in April, 12 individuals became ill and noroviruses (Genotype II) were found in one of the control meals. A second episode occurred in August: 50 people were affected and a combination of GI and GII noroviruses was found in one of the control meals analyzed. It is not clear what were the origin and the transmission route of the disease, but the food was a vehicle for its transmission to the different groups of this closed community.

A third outbreak happened in a hospital, where 17 out of 400 people became ill. Norovirus GII was detected in the soup as well as in 5 out of 6 faeces samples. For this outbreak too, it is not fully clear what the transmission route was, but the soup caused the infection to spread in the hospital. An infected person distributing the soup could have been the point of origin of the infection. Finally, noroviruses were found in 2.5% of the reported foodborne outbreaks in 2006 and accounted for 7.6% of the infections in humans. In all 3 outbreaks, noroviruses were detected in the analyzed food samples.

In 2007, 48 food samples from 11 foodborne outbreaks were suspected of being infected with noroviruses. For 8 of these outbreaks, there were samples screened for noroviruses because there was a food-handler involved. For the remaining 3 outbreaks, the reason was that the symptoms concerned were typical of norovirus infection and that there were no bacterial pathogens. Out of these 11 suspected foodborne outbreaks, the laboratory and epidemiological information confirmed that 10 were indeed foodborne norovirus outbreaks, whereas for the eleventh outbreak, the food and clinical specimens tested negative for the presence of noroviruses. Thus, 10 out of the 75 foodborne outbreaks that were reported in Belgium in 2007 were due to noroviruses. That is more than the number

of *Salmonella* outbreaks (8 reports). In total, 392 people were affected. In most cases, the symptoms appeared between 12 and 24 hours after food consumption. The symptoms reported generally concerned vomiting, diarrhoea and slight fever. Hospitalization was not necessary. Most outbreaks occurred at work (30%), the second most important settings were camps (20%) and nursing homes (20%). One outbreak took place in a restaurant (10%), one at a recreational park (10%) and one at home (10%).

In 8 outbreaks, the food-handler was suspected of being the source of the contamination. There weren't always any stool samples taken. In some cases, the stool samples were not screened for noroviruses but tested negative for bacterial pathogens. In several outbreaks, the food items concerned were handled and served by kitchen personnel and, according to the epidemiological information collected, the suspected source were the food-handlers. In two of those cases, there was a history of gastroenteritis reported in individuals involved in preparing the food: a member of the staff of a restaurant suffered from gastroenteritis the week before the outbreak and sandwiches prepared by the staff, including this particular food-handler, tested positive for noroviruses. On a camp, a sick child assisted in preparing sandwiches. In one outbreak after a school trip to a recreational park, noroviruses were detected both in leftovers of the served food (soup, chicken and rice) and in the human faecal samples. An infected person serving the meal for the children was probably responsible for contaminating the food. After the children had returned home, 34 more individuals became ill with the same symptoms as a result of satellite outbreaks in the families. In other outbreaks, mashed potatoes, meat stew and a composite meal tested positive, but no stool samples could be screened for noroviruses. Sandwiches were the vehicle of the norovirus outbreak in 40% of the cases. There was one suspected waterborne outbreak at a camping site in July. Epidemiological information pointed to tap water as the most likely source of the outbreak. However, due to the lack of an appropriate concentration/extraction method for noroviruses in water, the results obtained were negative.

Rotavirus

Rotavirus infection is the leading cause of severe acute diarrhoea among young children worldwide (Parashar *et al.*, 2006). The disease, which affects all age groups, is generally considered a mild infection in adults. The incubation period for rotavirus infection is 1 to 2 days. Typical symptoms are vomiting and watery diarrhoea, which develop quickly and persist for 3 to 8 days. Dehydration is a key factor that contributes to the high infant death rate, especially in developing countries where there is no good treatment available. An estimated 527 000 children under the age of 5 die from rotavirus diarrhoea each year, with over 85% of the deaths occurring in low income countries in Africa and Asia (Parashar *et al.* 2009). The WHO surveillance networks have revealed that between 2001 and 2008, approximately 40% of the hospitalizations for diarrhoea among children under the age of 5 were attributed to rotavirus infections.

The rotavirus A group could be further subdivided into G and P types on the basis of two outer capsid proteins VP7 and VP4. The most common strains are G1P, G2P and G9P (Anonymous, 2008).

As far as Europe is concerned, a recent study estimated that the annual rotavirus disease burden in the (at that time) 25 countries of the European Union involves 231 deaths and nearly 90 000 hospital admissions (Soriano-Gabarró *et al.*, 2006). Rotaviruses are transmitted by the faecal-oral route and the infection is not generally looked upon as foodborne. There have been some reports on outbreaks that were associated with food and water in a number of countries (Sattar *et al.*, 2001). In Italy, a large outbreak of viral gastroenteritis was caused by drinking water that was contaminated by a combination of noroviruses and rotaviruses. The source of the contamination could not be found, but extra chlorination of the water solved the problem (Martinelli *et al.*, 2007). In the Netherlands, poor food hygiene was identified as one of the major risk factors for rotavirus infection (De Wit *et al.*, 2003). 4 member states reported 127 rotavirus outbreaks to the EFSA in 2006. They affected a total of 568 people, 7% of whom were hospitalized. In Belgium, rotavirus infections are reported by the sentinel network (in 2007 in total 4194 cases), but there is no information available about related foodborne outbreaks.

Hepatitis E virus

Hepatitis E virus (HEV) is the etiological agent of non-HAV enterically transmitted hepatitis. It is the major cause of sporadic as well as epidemic hepatitis, which is no longer confined to Asia and the developing countries but has also become a concern in the developed nations. In the Indian subcontinent, it accounts for 30 – 60% of sporadic cases of hepatitis. It is generally acknowledged that hepatitis E is mostly self-limited and never progresses to become a chronic disease. The mortality is higher in pregnant women because the disease is aggravated by the development of fulminant liver disease (Panda *et al.*, 2007). HEV is predominantly transmitted by the faecal-oral route, although parenteral and perinatal routes have been implicated. The overall death rate among young adults and pregnant women is 0.5 – 3% and 15 – 20%, respectively (Cromeans *et al.*, 2001). The virus is not endemic in the western world. The first case of hepatitis E that was reported in the US was caused by travel to regions in which HEV is endemic. In countries in which HEV is not endemic, including the Netherlands, there have been few HEV infections reported that concerned individuals who had not travelled (Zaaijer *et al.*, 1993).

HEV was detected in pigs, where it was found to be able to replicate (Clayson *et al.*, 1995). According to recent evidence from Japan, HEV may be transmitted by the consumption of undercooked deer meat or pork. Sequence analysis showed that there is a 100% match between the strains isolated from contaminated deer meat and the patients (Tei *et al.*, 2003; Yazaki *et al.*, 2003). High antibody-positive rates have frequently been detected in domestic pigs and wild boars, including HEV genotypes 3 and 4, which suggests that those who eat uncooked meat are at risk of contracting HEV infection (Appleton *et al.*, 2007).

Some reports have very recently become available about chronic hepatitis E infections in patients with an immunocompromised status or in patients undergoing organ transplantation. In these cases, the patient was not reported to have travelled abroad recently (Colson *et al.*, 2008).

Hepatitis A virus

Hepatitis A occurs worldwide. In most cases, it is transmitted from person to person by the faecal-oral route. Infection is prevalent in settings with poor sanitary conditions. It is frequently asymptomatic in young children and its severity increases with age. In developing countries, more than 90% of children have been infected by the age of 6 (Cromeans *et al.*, 2001). Increasing general hygiene practices have led to reduced immunity among the population, which is now more prone to infections. Peak infectivity occurs during the 2 weeks that precede the onset of jaundice. With the first symptoms appearing several weeks after the infection, it could be transmitted by infected food-handlers through food. In Belgium, hepatitis A infections are reported by the sentinel network. 194 cases were notified in 2006. The number of infections remained stable in 2007, with 197 cases reported. By week 39 of 2008, a total of 265 cases of hepatitis A had been reported by the sentinel network. In September, 17 cases were notified in Brussels and a cluster of 6 cases occurred in St Jans-Molenbeek. The source, however, was not known. A large outbreak was reported by the Flemish health inspection in Limburg, where at least 48 individuals in total became ill after eating sandwiches prepared by an infected food-handler. An outbreak of HAV was described in Antwerp and Grimbergen in 2004. In total, 252 people became ill. The suspected source were food-handlers infected with hepatitis A who may have worked in a meat processing plant that supplied meat to butcher shops in the Antwerp and Grimbergen areas (de Schrijver *et al.*, 2004). 5 European countries reported outbreaks caused by the hepatitis A virus to the EFSA in 2006. In total, 39 outbreaks were reported, affecting 181 people, of whom 38.1% were hospitalized. In USA, hepatitis A is said to be the most common cause of hepatitis, with a reported rate of 0.3%. Each year, some 30 – 50 000 cases of hepatitis A occur in USA (Fiore, 2004). Contaminated food is a common vehicle of transmission of hepatitis A. In addition to infected food workers, fresh produce contaminated during cultivation, harvesting, processing, and distribution has also been known to be a source of hepatitis A (Fiore, 2004). In 1997, frozen strawberries were found to be the source of a hepatitis A outbreak in five states (Hutin *et al.*, 1999), and in 2003, fresh green onions were identified as the source of a hepatitis A outbreak that was traced down to the consumption of food at a restaurant in Pennsylvania (Wheeler *et al.*, 2005).

Sapovirus

The prototype strain of human SaV, the Sapporo virus, was originally discovered during an outbreak in an orphanage in Sapporo, Japan, in 1977 (Chiba *et al.*, 1979). SaV can be divided into five genogroups (GI-GV), of which GI, GII, GIV and GV are known to infect humans, while SaV GIII infects porcine species.

SaV can cause sporadic cases of acute gastroenteritis that require hospitalization, as well as symptomatic and asymptomatic infections that don't (Hansman *et al.*, 2004; Okada *et al.*, 2002; Vinje *et al.*, 2000; Jiang *et al.*, 1999; Pang *et al.*, 2000). SaV infection is more frequent in young children than in adults and almost always occurs by the age of 5 (Hansman *et al.*, 2007a). In addition, children at day-care centres and institutions such as elementary schools are at greatest risk of contracting and transmitting SaV-associated infections.

There have only been a limited number of studies on SaV. It has therefore been difficult to look for correlations between the rates of incidence, detection and overall prevalence or to draw conclusions on them (Hansman *et al.*, 2007a). The rates of incidence, detection and overall prevalence of SaV infections vary from one country to another and are likely to be affected by the diagnostic techniques used (Lopman *et al.*, 2003). The method that is currently the most widespread is reversed transcription-PCR (RT-PCR) (Okada *et al.*, 2002; Vinje *et al.*, 2000; Jiang *et al.*, 1999). More recently, there has been a novel, TaqMan-based real-time RT-PCR method developed (Oka *et al.*, 2006).

A number of reports have noted that SaV detection rates were usually much lower than norovirus detection rates (Buesa *et al.*, 2002; Pang *et al.*, 2000; Kirkwood *et al.*, 1999; Wolfaardt *et al.*, 1997). In addition, SaV gastroenteritis appears to induce symptoms that are milder than those caused by noroviruses, which often makes hospitalisation unnecessary (Pang *et al.*, 2000; Kirkwood *et al.*, 2001; Sakai *et al.*, 2001).

There have recently been several important findings human SaV. SaV strains have been identified in water samples, which included samples from both untreated and treated wastewater and from river water (Hansman *et al.*, 2007b). SaV strains were also detected in shellfish samples destined for human consumption. Also, recombinant SaV strains were identified in a number of different countries. This suggests that SaV contaminations in the natural environment may lead to foodborne infections in humans. However, further studies are needed to determine exactly how this can cause gastroenteritis in humans.

Chapter IV – VIRUS STABILITY AND INACTIVATION

The resistance of viruses in the environment

Viruses do not replicate in the environment outside living cells. They can be subdivided into three classes depending on their resistance to the environment and to biocides:

Class A: lipid-containing viruses (enveloped viruses) (e.g. *Flaviviridae*, *Orthomyxoviridae*); ex. TBEV, influenza A virus, hantavirus, coronavirus, rabies virus, Nipah virus;

Class B: small naked viruses (20 – 30 nm diameter), (non-enveloped) (e.g. *Picornaviridae*, *Caliciviridae*, *Astroviridae*); ex. noroviruses, sapoviruses, HAV, HEV, aichiviruses;

Class C: other naked viruses (of greater size), (non-enveloped) (e.g. *Adenoviridae*, *Reoviridae*); ex. rotaviruses, adenoviruses, reoviruses (Maris, 1995).

It follows that most foodborne viruses are non-enveloped and exhibit a high resistance to the physico-chemical conditions of the environment.

The non-enveloped viral particles cannot be inactivated with chloroform, lipid solvents and detergents. Their stability in the environment is determined by several parameters, which include pH-values, sunlight, humidity and protein concentration. Heat treatments (85 to 90°C during 1:30 min) inactivate viruses in shellfish. The temperature is therefore an important parameter, with low temperatures enhancing virus resistance.

The nature of the virus determines which disinfection method will be used. For complete disinfection from all foodborne viruses, it is advised to resort to the most effective disinfectants, which are active against the most resistant viruses, i.e. picornaviruses and calciviruses. Disinfectants can only be used successfully if they are part of a general procedure that includes pre-disinfection cleaning. In fact, with extraneous organic material diluting and quickly neutralising biocidal chemicals, it is the most important factor in the outcome of any disinfection operation (Kahrs, 1995).

The persistence of viruses in food

Viruses that belong to the *Picornaviridae* family can persist well on fresh produce (Table 3). Thus, there was no reduction of foodborne viruses observed in most vegetables after one to two weeks storage. Some vegetables, such as carrots and fennel, are exceptions. It is suggested that antimicrobial substances in these vegetables may be responsible for the observed decline (Rzezutka and Cook, 2004). As human NoV cannot be cultivated, survival/inactivation experiments are carried out with a surrogate. FCV and MuNV are used as surrogates for human NoV. FCV is a member of the *Vesivirus* genus and causes an oral and respiratory

illness in cats. MuNV belongs to the same genus as the human NoV and is asymptomatic in wild type mice. With FCV less stable than HAV or poliovirus, MuNV is believed to be a better model for human NoV. Though there are less data available for MuNV, the limited findings there are reveal that MuNV is more stable than FCV.

All viruses survive longer at 4°C than at room temperature. Unfortunately, 4°C is the most frequently used temperature for the storage of fresh produce.

The inactivation of viruses in food

Heat-inactivation is a widely used method for micro-organisms in the food industry. Many inactivation rates are reported for HAV. They reveal that the inactivation profile is dependent upon the matrix. HAV is more resistant in strawberry mashes (Deboosere *et al.*, 2004) than in milk (Bidawid *et al.*, 2000a). Comparing the results of different studies is not always justified because different experimental set ups could be responsible for the observed differences. Consequently, inactivation rates cannot easily be extrapolated to another matrix. These studies indicate that adequate heating such as boiling or classical pasteurization will at least achieve a 3 log reduction.

High hydrostatic pressure (HHP) has emerged as a promising nonthermal technology for pasteurizing food products (Kingsley *et al.*, 2007). HHP technology is of interest for the seafood industry in particular. Shellfish are consumed uncooked, which means that there can be no heat treatment used. HHP retains the appearance, flavour and texture of the food (Murchie *et al.*, 2005). It is effective against HAV and MuNV (Table 4). However enteroviruses seem to be very resistant to HHP. Although enteroviruses belong to the same family as HAV, the inactivation rates that were observed were totally different. These findings stress the need to investigate the behaviour of different viruses (Koopmans and Duizer, 2004).

Food processing industries dealing with fresh produce often have a decontamination procedure in place (water, sodium hypochlorite, peroxyacetic acid). Washing does not substantially remove viruses. Peroxyacetic acid (300 ppm) yields higher reductions than sodium hypochlorite (800 ppm). Despite the poor removal/inactivation of enteric viruses on fresh produce, sanitizers are needed to maintain the microbiological quality of the wash water, thus preventing cross-contamination from the water to the washed fresh produce.

The data presented in table 3 indicate that picornaviruses are more resistant to gamma irradiation than calciviruses and MS2 bacteriophages, although picornaviruses were inactivated more quickly after UV treatment than calciviruses and MS2 bacteriophages. Gamma irradiation was found to be greatly affected by the presence of proteins (De Roda Husman *et al.*, 2004).

Foodborne viruses can withstand low pH-values. These viruses need to pass the stomach, an acid environment, before reaching the intestines and causing

gastroenteritis in humans. This acid stability can account for the fact that raspberries contaminated with enteric viruses have been implicated in foodborne outbreaks (Cotterelle *et al.*, 2005).

TABLE 3.
Virus persistence according to temperature and matrix

Virus		Conditions		Survival/reduction		Matrix		Reference	
Reoviridae	rotavirus	4°C, 3 days		3		Filtered fruit juice (pH 2.98)		Mahony <i>et al.</i> , 2000 Badawy <i>et al.</i> , 1985 Sattar <i>et al.</i> , 1984	
	rotavirus SA-11	4°C		30, 30, 25 days		Lettuce, radishes, carrots			
	rotavirus	RT ^e		25, 4, 15 days		aerosols			
	HAV ^a	9 days at 20°C		No reduction		lettuce		Croci <i>et al.</i> , 2002	
		4°C, 9 days		> 2.44		Carrot			
		4°C, 4 days		> 3		Fennel			
	HAV	4 weeks		0.4		Marinated mussels		Hewitt and Greening, 2004	
						Acid marinade (pH =3.75)			
	Picornaviridae	HAV	85°C <0.5 min		5		milk		Bidawid <i>et al.</i> , 2000a Deboosere <i>et al.</i> , 2004 Croci <i>et al.</i> , 1999 Pary and Mortimer, 1984
			80°C 0.68 min (skimmed), 1.24 min (cream)		5		milk		
		85°C 2.37 min (40° Brix); 4.98 min (52° Brix)		1		1 g strawberry mashes			
		80°C 8.94 min (52° Brix)		>4.6; >4.6		4 ml virus suspension			
		60°C 10 min; 80°C 3 min		2; 2		4 ml shellfish homogenate			
		60°C 10 min; 80°C 3 min		2, 3		PBS			
		65°C 2 min, 65°C 4 min		3; 2		milk			
		62.8°C 30 min; 71.6°C 15 s		0.56; >5		milk			
poliovirus		72°C 15 s; 72°C 30 s		0.41; >5		yoghurt			
poliovirus		42°C 30 h; 55°C 30 min		13 days		Spinach			
poliovirus	2 h irrigation and direct harvesting		19% reduction		Fresh green-lipped mussels		Ward and Irving 1987		
poliovirus	4°C, 2 days		1		Lettuce		Greening <i>et al.</i> , 2001		
poliovirus	4°C, 11.6 days						Kurdziel <i>et al.</i> , 2001		

TABLE 3.
Virus persistence according to temperature and matrix

Virus	Temperature	Duration	Reduction	Matrix		Reference
				Matrix 1	Matrix 2	
Picornaviridae	poliovirus	4°C, two weeks	No reduction	Green onions		Tiron, 1992; Rzezutka and Cook, 2004
		4°C, 14.2 days	1	White cabbage		
		4°C, two weeks	No reduction	Fresh raspberries		
		-20°C, 8.4 days		Frozen strawberries		
		4°C	120 days, 120 days	Raw milk, yoghurt		
	poliovirus, coxsackievirus	4°C	90 days	yoghurt		Konowalchuk and Speirs, 1975
	coxsackievirus B5	4°C, 16 days	No reduction	Moist conditions, lettuce		
	FCV ^b	4 weeks	8	Marinated mussels		Hewitt and Greening, 2004
	FCV	30 s immersion in boiling water	2	6-8 cockles		Slomka and Appleton, 1998
	Caliciviridae, FCV, CaCV	5 cycles of freeze/thawing	0.44; 0.34	Cell culture medium		Duizer et al., 2004a
	MuNVc; FCV	25°C	0.13; 0.40	Surface water		Bae et al., 2008
	MuNV; FCV	RT, 7 days	1; 4	Faecal matrix		Cannon et al., 2006
	FCV, CaCV ^d	20°C, 1 week	3	Cell culture medium		Doultree et al., 1999
	FCV	4°C, 7 days ; RT, 4 days	2, > 3	Lettuce		Mattison et al., 2007a Doultree et al., 1999
		4°C, 6 days ; RT, 2 days	3, 3	Slices strawberries		
		4°C, 7 days ; RT, 7 days	1, 1	Ham		
		4°C	> 60 days	in suspension		
	NoV	-	Incomplete	Stool filtrate		Dolin et al. 1972; Koopmans and Duizer, 2004
	MuNV	80°C 2.5 min	6.5	Cell culture medium		Baert et al., 2008a
Leviviridae	MS2 coliphage	4°C, 7 days	< 1	Lettuce, cabbage, tomato, onions,....		Dawson et al., 2005

^aHAV: hepatitis A virus; ^bFCV: feline calicivirus; ^cMuNV: murine norovirus; ^dCaCV: canine calicivirus; ^eRT: room temperature

TABLE 4.**Virus inactivation depending on physico-chemical conditions**

Virus		Inactivation method	Log ₁₀ reduction	Matrix	Reference
High hydrostatic pressure					
Adenoviridae	adenovirus	400 MPa, 20°C, 15 min	5	Cell culture medium	Wilkinson <i>et al.</i> , 2001
Reoviridae	rotavirus	300 MPa, 25°C, 2 min	8	Cell culture medium	Khadre and Yousef, 2002
	HAV ^a	450 MPa, ambient temp, 5 min	> 6	Cell culture medium	Kingsley <i>et al.</i> , 2002
	HAV	400 MPa, 20°C, 5 min	> 3	Seawater	Kingsley <i>et al.</i> , 2002
	HAV	375 MPa, 21°C, 5 min	4.3; 4.7	Mashed Raspberries; sliced green onions	Calci <i>et al.</i> , 2005
Picornaviridae	poliovirus	600 MPa, ambient temp, 5 min	No red ^e	Cell culture medium	Kingsley <i>et al.</i> , 2004
	aichi virus	600 MPa, 20°C, 60 min	No red		Wilkinson <i>et al.</i> , 2001
	coxsackie virus B5	600 MPa, ambient temp, 5 min	No red		
	coxsackie virus A9	600 MPa, ambient temp, 5 min	7-6	Cell culture medium	Kingsley <i>et al.</i> , 2004
Caliciviridae	FCV ^b	275 MPa, ambient temp, 5 min	> 6	Cell culture medium	Kingsley <i>et al.</i> , 2002
	FCV	200 MPa, -10°C or 50°C, 4 min	5 or 4	Cell culture medium	Chen <i>et al.</i> , 2005
	MuNV ^c	400 MPa, 5°C, 5 min	4	Cell culture medium	Kingsley <i>et al.</i> , 2007
	MuNV	450 MPa, 20°C, 5 min	6.85	Cell culture medium	Kingsley <i>et al.</i> , 2007
Washing with water and the use of chemical agents					
Picornaviridae		water	No red	salads, fruits (strawberries)	Mariam and Oliver, 2000; Koopmans and Duizer, 2004
	HAV	water: 5 min	0.1	10 g lettuce/100ml water	
		water: 5 min	1	10 g fennel/100ml water	
		water: 5 min	0.9	10 g carrot/100ml water	
Caliciviridae		PAA ^g 300 ppm; 150 ppm 10 min	3 ⁱ ; 1 ^f	100 g strawberries/ 100 ml sanitizer solution	
		PAA 300 ppm; 150 ppm, 10 min	3 ⁱ ; 2 ^f	10 g lettuce/100 ml	Gulati <i>et al.</i> , 2001

TABLE 4.

Virus inactivation depending on physico-chemical conditions

	water 10 min	2	strawberries and lettuce	
FCV	NaOCl 200 ppm; 800 ppm, 10 min	0; 1.5	lettuce	Allwood <i>et al.</i> , 2004
	bleach 50 ppm, 100 ppm, 200 ppm	2.2, 2.6, 2.9	3cm ² disks of lettuce in 5 ml	
	PAA 80 ppm	2.9	sanitizer solution, 2 min	
	3% H ₂ O ₂	2.8		
	NaOCl 300 ppm 10 min	2	Cell culture medium	
NaOCl 300 ppm 10 min	> 3	Cell culture medium		
CaCV	Water 0.42 min	0.39	2 onion bulbs/100 ml	Baert <i>et al.</i> , 2008b
	Water 2 min	1.01	10 g spinach leaves/350 ml	
	Water 5 min	1.14	50 g lettuce/500 ml	
	NaOCl 200 ppm 5 min	1.04 ^g	50 g lettuce/500 ml	
	PAA 80 ppm; 250 ppm 5 min	0.77 ^g ; 1.43 ^g	50 g lettuce/500 ml	
MS2	water 5 min	< 0.5	100 g/11 lettuce	Dawson <i>et al.</i> , 2005
	chlorine 100ppm, 5 min	0.7		
	bleach 50 ppm; 100 ppm, 200 ppm	1.9, 2.7, 2.9	3cm ² disks of lettuce in 5 ml	Allwood <i>et al.</i> , 2004
	PAA 80 ppm	2.8	sanitizer solution, 2 min	
	3% H ₂ O ₂	2.6		
Acidity				
Reoviridae	rotavirus	inactivated		Weiss and Clark, 1985; Seymour and Appleton, 2001
Picornaviridae	HAV	survival		Dolin <i>et al.</i> , 1972
	HAV	incomplete		Hollinger and Ticehurst 1996; Koopmans and Duizer, 2004
	HAV	Still infectious		Scholz <i>et al.</i> , 1989

^aHAV: hepatitis A virus; ^bFCV: feline calicivirus; ^cMuNV: murine norovirus; ^dCaCV: canine calicivirus; MS2: ^eNo red: no reduction; D_{85°C}: time (min) needed to cause one log reduction at 85°C; ^gPAA: peroxyacetic acid

TABLE 4.

Virus inactivation depending on physico-chemical conditions

Virus		Inactivation method		Log ₁₀ reduction		Matrix		Reference	
Acidity									
Caliciviridae	FCV, CaCV	pH 2 or > 10		5				Duizer et al., 2004a	
	NoV	pH 2.7, 3h		incomplete				Dolin et al., 1972; Koopmans and Duizer, 2004	
	MuNV	pH2		<1			Cell culture medium		
Irradiation									
Picornaviridae	HAV	High intensity broad spectrum pulsed light 0.5 J/cm ² ; 1 J/cm ² ; 2 J/cm ²		2.1; 4.1; >5.6			PBS + 5% FCS	Roberts and Hope, 2003	
	HAV	3 kGy		1			lettuce, strawberries (tested with 1 piece)	Bidawid et al., 2000b	
	poliovirus	High intensity broad spectrum pulsed light 0.5 J/cm ² ; 1 J/cm ² ; 2 J/cm ²		1.9; 3.2; > 5.5			PBS + 5% FCS	Roberts and Hope, 2003	
	coxsackie virus B2	7 kGy		1			ground beef	Sullivan et al., 1973	
	FCV	UV 120 J/m ² , 500 Gy		3			virus suspension, no diff low or high protein content for UV in contrast to gamma radiation!	de Roda Husman et al., 2004	
Caliciviridae	CACV	UV 200 J/m ² , 300 Gy		3					
Leviviridae	MS2	UV 650 J/m ² , 100 Gy		3					
Packaging									
Picornaviridae	HAV	CO ₂ : N2 70:30, RT for 12 days		43% survival			MAP lettuce	Bidawid et al., 2001	

^aHAV: hepatitis A virus; ^bFCV: feline calicivirus; ^cMuNV: murine norovirus; ^dCaCV: canine calicivirus; MS2 : ^eNo red: no reduction; ^fD_{85°C}: time (min) needed to cause one log reduction at 85°C; ^gPAA: peroxyacetic acid

Chapter V - PREVENTION

Hygienic and technological prevention of viral food chain contamination

In contrast to bacteria, viruses cannot replicate in food or water. Thus, the number of infectious virus particles will not increase during storage. Virus stability, the food processing method used, the initial level of contamination, the infectious dose and host susceptibility will determine whether or not food that is contaminated with viruses will serve as a vehicle of human infection (Koopmans *et al.*, 2002). Viruses can withstand extreme environmental conditions, such as low pH values. It follows that they are able to survive many food and storage conditions.

The faecal-oral route is the most common mode of transmission for foodborne viruses. There can be more than a million virus particles/ml in the faeces of infected individuals. Therefore, Good Hygienic Practices (GHP) form the basis of primary prevention. Personal and collective hygiene starts with washing one's hands before each meal and after visiting the toilet. Food-handlers play an important role in the transmission of viruses/the contamination of food products and must be informed about the faecal-oral risk and food hygiene measures. They must receive vaccination (HAV, rotavirus, enterovirus). Also, they must be prohibited from handling food if they have any symptoms of gastroenteritis or hepatitis. The observance of cleaning-disinfection protocols and the choice of primary materials contribute towards improving viral risk control.

Food products that pose a high risk of contamination with human enteric viruses can be categorized into different groups: (1) shellfish, (2) fresh produce (soft fruits and vegetables) and (3) ready-to-eat (RTE) food products. For each of these food products, the prevention of foodborne infection with human enteric viruses will be carried out differently.

Besides preventing the viral contamination of food products countering the secondary spread of these viruses and the emergence of an outbreak is a priority. The aim is to limit the number of infected individuals.

Shellfish

Shellfish, which are often eaten raw or slightly cooked, pose a high risk for infection as they are potential vehicles for enteric viruses. From an epidemiological point of view, HAV and NoV have been linked to viral disease associated with shellfish. Shellfish are most commonly contaminated by being grown in faecally polluted water at the pre-harvest level. Filtering molluscs that live in contaminated waters concentrate viruses at high levels in their hepatopancreas (Richards, 2001). The extent to which viruses are accumulated in shellfish depends on the hydraulic characteristics of the exposure system, the type of virus, the concentration of virus in the water, the temperature, the pH-value, salinity, and the presence of particles in the water (Sobsey and Jaykus, 1991).

Conventional faecal indicators are not reliable to show the presence or absence of NoVs. It is dangerous to eliminate faecal bacterial indicators in order to determine the purification times of the molluscs. The use of *E.coli* instead of faecal coliforms is recommended to test the quality of batches of cooked shellfish products. It seems to be of crucial importance to develop methods of analysis before setting up criteria that apply to pathogenic viruses in live shellfish (Regulation (EC) No 2073/2005, preamble 12). A working group is now validating a horizontal method for the detection and determination of NoV and HAV in food by RT-PCR.

The most effective strategy to prevent the viral contamination of shellfish is to harvest them in areas with good quality water that is free from human sewage. Only shellfish that come from controlled and clean harvesting areas must be eaten. Depuration refers to the reduction of contaminating microorganisms by placing shellfish in clean, often disinfected, seawater under specific conditions. Disinfection is only carried out upstream harvesting areas, which means that the biological depuration cycles of sewage do not destroy HAV. Ozone exhibits a greater virucidal efficiency than chlorine: 0.4 mg/L ozone induce a 2 to 4 Log₁₀ reduction in the virus population after a 4-minute contact. The action of chlorine on viruses depends on the pH-value. It is more effective on bacteria. Consequently, both ozone and chlorine processes are often used to disinfect water. Relaying, also known as natural purification, is the transfer of shellfish to approved areas. Depuration has been shown to be successful in reducing bacterial disease associated with shellfish consumption. However, whilst (enteric) bacteria are rapidly reduced (within 48h), viruses may persist for several days (Richards, 2001). Depuration seems less effective in eliminating viruses than bacteria and should only be used for shellfish that are only slightly contaminated (Richards, 2001). Ionizing radiation has also been investigated as a means of inactivating enteric viruses in shellfish (Mallet *et al.*, 1991). NoV inactivation requires UV doses over 10³ mJ/cm². This process is often applied in depuration plants, where irradiation doses of approximately 25mJ/cm² are used. This is close to the dose required for the potabilisation of water according to European standards.

The systems operate either in open or closed circuit, and are often preceded by sand filters or settling tanks in order to reduce the water turbidity when necessary, thereby optimizing treatment quality. The equipment is usually sized on the basis of a flow rate of 10 m³/h/t of shellfish depurated, in continuous flow. The results obtained from monitoring depuration plants show that UV radiation is very effective (Muniain-Mujika *et al.*, 2002). This treatment can thus be regarded as a good alternative to other conventional treatments, thanks to the low investment and operating costs involved, easy maintenance, environmental safety and small size requirements.

Human enteric viruses are quite resistant to traditional food conservation methods such as cooling and freezing. It follows that they cannot be controlled effectively by means of these processes (Lees, 2000). The types of virus and matrix have been shown to play an important role in the heat sensitivity of the virus. The density of the shellfish tissue and the concentration of the virus in the digestive tract reduce heat

penetration, which means that longer heat treatments are needed to inactivate HAV in shellfish than in buffer (Crocì *et al.*, 1999). Noroviruses are resistant to heat (37°C during 120 hours or 100°C during 1 minute). This is also the case with HAV (60°C during 1 hour, partially inactivated after 10 to 12 hours at the same temperature). Virus inactivation in shellfish requires cooking with a heart temperature of 90°C for 2 minutes.

There have been non-thermic processes suggested for the inactivation of HAV and NoVs in shellfish, such as high hydrostatic pressure (Kingsley *et al.*, 2002). Viruses have demonstrated a wide range of sensitivities in response to high hydrostatic pressure (Grove *et al.*, 2006). This process has recently been applied by some industries.

Because of the severity of HAV infection, it is best to advise immuno-compromised patients to avoid this type of food product (Potasman *et al.*, 2002).

Raw fruit and vegetables

Over the last 20 years, there have been many reports of viral foodborne outbreaks that were induced by the consumption of contaminated raw fruit and vegetables. The most common viral agents associated with fresh produce are HAV and noroviruses. Raw fruit and vegetables can be contaminated before the food product reaches food service establishments (Koopmans *et al.*, 2002). Contaminated soil, irrigation or washing water and infected food-handlers are all possible sources of contamination. Sewage sludge treatment (e.g. by drying, pasteurization, composting) can reduce viruses, but it cannot eliminate them. Therefore, the use of recycled sewage effluent and sludge for the irrigation or fertilization of crops involves the risk of virus contamination. The use of contaminated water for spray irrigation is very risky (Seymour and Appleton, 2001). Fresh produce with a rough or irregular surface in particular may pose the greatest problem, because faecal matter and organic material can easily adhere to it. The persistence of viruses on fresh produce is also dependent on pH-values, moisture content and temperature. Preharvest control strategies aimed at reducing enteric viruses in fresh produce must take into consideration their production, packaging and transport based on GAP and GMP. Primary products and raw material must be protected from contamination by humans, domestic animals and agricultural waste that is a known source of viruses/micro-organisms (Koopmans and Duizer, 2004). Many fresh fruits and vegetables undergo extensive human handling during harvesting, so preharvest control must focus on food-handlers as well. All personnel, including seasonal workers, should have a good knowledge of basic hygiene principles and should report any case of illness to their supervisors (Koopmans *et al.*, 2002; Koopmans and Duizer, 2004). Food-handlers with symptoms that are consistent with exposure to infectious foodborne diseases must be excluded from work until 48h after recovery. When they resume work, these food-handlers need to follow strict hygiene rules, as they may shed substantial numbers of NoV for weeks (Koopmans and Duizer, 2004).

Many food products are washed before they enter the distribution chain. Wash water must be clean or disinfected with chlorine or an alternative sanitizer. Generally, washing with plain water reduces the virus presence by about 90%, but this reduction depends on several factors, such as the type of food, the type of virus, the level of contamination and the water temperature. In the EU, decontamination aimed at reducing the microbial load on foods is forbidden. Water that comes into contact with food must be of drinking water quality. If the water does not comply with the microbiological, chemical and physical parameters defined in the Royal Decree of 14/01/2002, it must be disinfected by means of e.g. ozone, UV, chlorine or other techniques such as ultrafiltration. If chlorine or other technical additives are used, this procedure should be validated against the presence of chemical residues. In addition, there should be no remaining chemical residues from the wash water on food products.

Ultraviolet radiation has recently been suggested as an alternative to chemical methods for the disinfection of water. The UV doses needed for a $3 \log_{10}$ reduction of FVC and enteric adenovirus type 40 were 21 and 153 mJ/cm^2 , respectively (Thurston-Enriquez *et al.*, 2003).

Contamination of ready to eat (RTE) food occurs after it has been processed by food-handlers. Most documented viral foodborne outbreaks can be traced down to food that has been manually handled by an infected person, rather than to industrially processed foods (Koopmans and Duizer, 2004). Food-handlers may transmit enteric viruses from contaminated surfaces, from food or from contaminated hands. Human faecal material is the most important source of enteric viral contamination, as it can contain millions of viral particles, but vomit may also contain infectious viruses and is therefore a potential source of contamination. In the case of vomit, secondary spread is more important, as aerosol formation can lead to exposed individuals being infected. Food-handlers need to be educated specifically about the microbial safety guidelines and hygiene rules. This includes their being educated about the risk of exposure to viruses through sick children in their households (Koopmans and Duizer, 2004). As already mentioned above, food-handlers with symptoms of foodborne enteric disease must be excluded for 48 hrs after recovery and need to follow strict hygiene rules when they return to work (Koopmans and Duizer, 2004).

Stringent personal hygiene during food preparation is very important to prevent the contamination of RTE foods (Koopmans and Duizer, 2004). The hands are believed to play a key role in the spreading of viruses. Thus, hand hygiene is crucial to prevent the contamination of RTE foods. Successfully controlling viral foodborne disease outbreaks requires an effective disinfecting product and adequate user instructions. Interactive training is also recommended (Lilquist *et al.*, 2005). A product is generally considered effective if it can reduce the virus titre by at least $3 \log_{10}$. The activity of antiseptic hand cleansers against bacteria may not reflect the ability of these products to eliminate viruses.

Mbithi *et al.* (1993) tested 10 hand-washing agents against HAV and poliovirus, none of which turned out to eliminate these viruses appropriately ($>3 \log_{10}$ reduction). Sattar *et al.* (2002) tested several antiseptics against rotavirus and HAV and found that only a formulation containing 75% ethanol resulted in a $3 \log_{10}$ reduction of HAV, while none of the products led to effective rotavirus elimination. Gehrke *et al.* (2004) observed the highest reduction of FCV ($3-4 \log_{10}$) with 70% ethanol or 1-propanol. In contrast, Lages *et al.* (2008) found antiseptics containing 1% available iodine to be more effective against FCV (2.67 log reduction) than alcohol-based sanitizers (only 1.3 log reduction). Bidawid *et al.* (2004) suggested to use ethanol-based hand rubs only to decontaminate the hands between hand-washing, as water and soap seemed to be more effective in reducing FCV spread. Thus, since no hand sanitizer (including alcohol-based hand disinfectants) was shown to eliminate enteric viruses from the hands effectively, hand sanitizers must not be used instead of proper hand washing.

Hot-air drying also seems to play a crucial role in removing the viruses from the hands. It has been shown to be more effective against rotavirus than drying hands with paper or cloth towels (Ansari *et al.*, 1991). The use of automatic or foot-controlled faucets may also reduce the likelihood of recontaminating one's hands after washing. It is difficult to thoroughly and repeatedly disinfect one's hands with chemicals, as this can damage the skin. The use of disposable gloves is a good alternative to frequent hand washing and disinfection.

Cleaning and disinfecting surfaces is highly important to prevent enteric viral disease because viruses can be transmitted to hands or food upon contact with contaminated surfaces.

It has been concluded that human enteric viruses have a mean persistence of approximately 2 months (Kramer *et al.*, 2006). Thus, enteric viruses seem to survive very well. As a result, they can be a continuous source of transmission unless surfaces are regularly disinfected. The persistence of human enteric viruses on surfaces depends on several factors, such as virus type, type of surface, temperature and relative humidity. Low temperature is mostly associated with a longer persistence (Mbithi *et al.*, 1991). Table 5 provides a summary of several inactivation studies. As is the case with hand hygiene products, surface disinfection is effective if it leads to a \log_{10} reduction of at least 3. Many surface disinfectants do not successfully inactivate enteric viruses. Sodium hypochlorite at a concentration of 1 000 ppm (corresponding to 16 ml bleach (eau de Javel) (20°) per litre of water) has been shown to be effective for the inactivation of HAV (Jean *et al.*, 2003; Terpstra *et al.*, 2007) and FCV (Jimenez *et al.*, 2006). Quaternary ammonium compounds also seem useful to inactivate HAV and FCV (Mbithi *et al.*, 1990; Gulati *et al.*, 2001; Jean *et al.*, 2003; Jimenez *et al.*, 2006), though the former appear to require higher concentrations.

TABLE 5.
Effectiveness of disinfectants for inactivating human enteric viruses on different types of surfaces

Virus	Surface type	Agent/ concentration	Contact time	Log ₁₀ reduction	Reference	
HAV	Stainless steel disks	2% glutaraldehyde	1 min	>4	Mbiti <i>et al.</i> , 1990	
		sodium hypochlorite (5 000 ppm free chlorine)	1 min	>4		
		quaternary ammonium formulation containing 23% HCl	1 min	>4		
		phenolics	1 min	<1		
		iodine-based products	1 min	<1		
		alcohols	1 min	<1		
		solutions of acetic, peracetic, citric and phosphoric acid	1 min	<1		
	Stainless steel, aluminium, polyvinyl chloride, high-density polyethylene, copper		quaternary ammonium glutaraldehyde (3 000 ppm)	5 min at 4°C or 1 min at 22°C	<3	Jean <i>et al.</i> , 2003
			quaternary ammonium glutaraldehyde (1 000 ppm)	5 min at 22°C	>3	
			sodium hypochlorite (1 000 ppm)	5 min at 4°C or 1 min at 22°C	<3	
Stainless steel		sodium hypochlorite (1 000 ppm)	5 min at 22°C	>4	Terpstra <i>et al.</i> , 2007	
		sodium hypochlorite (3 000 ppm)	1 min at 4°C	<3		
		sodium hypochlorite (3 000 ppm)	5 min at 4°C or 1 min at 22°C	>3		
		quaternary ammonium (500 ppm)	5 min at 22°C	>5		
		0.1 N sodium hydroxide	10 min	3		
		sodium hypochlorite (1 000 ppm)	1 min	>5		

TABLE 5.

Effectiveness of disinfectants for inactivating human enteric viruses on different types of surfaces

HAV Rotavirus	polystyrene	30% sodium chlorite	1 min (28°C)	<3; <2	Abad <i>et al.</i> , 1997
		70% ethanol	1 min (28°C)	<2; <1	
		0.05% chlorhexidine digluconate	1 min (28°C)	<1; <1	
		0.125% sodium hypochlorite	1 min (28°C)	<2; <2	
		1.41% phenol+ 0.24% sodium phenate	1 min (28°C)	<2; <1	
		0.0192% diethylenetriamine	1 min (28°C)	<2; <2	
Rotavirus	Stainless steel disks	0.1% o-phenylphenol/79% ethanol	10 min	>4	Sattar <i>et al.</i> , 1984
		6% sodium hypochlorite (800 ppm free chlorine)	10 min	<2	
		7.05% quat diluted 1:128 in tap water	10 min	<1	
		14.7% phenol diluted 1:256 in tap water	10 min	<2	
		n-quaternary ammonium compound (1 800 ppm)	10 min	<3	
		n-quaternary ammonium compound (1 560 ppm) + 0.0625% sodium bicarbonate	10 min	>3	
FCV	Stainless steel disks	sodium hypochlorite (800 ppm)	10 min	<2	Gulati <i>et al.</i> , 2001
		sodium hypochlorite (5 000 ppm)	10 min	>3	
		0.03% peroxyacetic acid + 0.022% hydrogen peroxide	10 min	3	
		iodine + phosphoric acid (300 ppm iodine)	10 min	2	
		0.037% o-benzyl p-chlorophenol + 0.037% o-phenylphenol	10 min	>6	
		quaternary ammonium (850 ppm)	10 min at 20°C	>6	
		sodium hypochlorite (100 ppm)	10 min at 20°C	>3	
		sodium hypochlorite (1 000 ppm)	10 min at 20°C	>6	
		ethanol (70%)	1 min	2	
		isopropanol (40-60%)	1 min	2	
polystyrene	Stainless steel	metricid (phenolic compound)	1 min	99.99%	Malik <i>et al.</i> , 2006b
			10 min	red	
Fabrics	Carpets	metricid (phenolic compound)	1 min	99.99%	Malik <i>et al.</i> , 2006a
			10 min	red	

Medical prophylaxis (vaccination)

Prophylactic measures against virus diseases are essentially aimed at food producers and food-handlers. Consumer related hygiene is more important for the prevention of bacterial disease and toxin related illness following the consumption of contaminated food.

General hygiene measures have been dealt with in detail above. It is also important to remove food-handlers who show symptoms that point to a risk of contaminating the food with viruses, i.e. symptoms of gastrointestinal disease (vomiting or diarrhoea) or hepatitis.

There are different vaccines available against the viral diseases that were discussed in this report, including the poliomyelitis vaccine, TBE vaccine, rotavirus vaccine and hepatitis A vaccine. However, only the hepatitis A vaccine is indicated as a general measure for all those who work in food production or who manipulate unwrapped foods.

Recommendations about this vaccine, which is administered as a two-dose schedule, were published by the Superior Health Council of Belgium in 2007 (www.health.fgov.be/CSS_HGR/; see: vaccination fact sheets CSS-HGR 8205, February 2007 - Vaccination of adults against Hepatitis A: 28-29).

Chapter VI - RISK ASSESSMENT: THE STATE OF THE ART

Introduction

Risk assessment is one of the three components of the risk analysis framework. It has been defined by the *Codex Alimentarius* (FAO/WHO, 1995) as typically consisting of several distinct steps: first, hazard identification, second, exposure assessment and hazard characterization, and finally risk characterization, which eventually identifies and preferably quantifies the risk.

Definitions:

Risk assessment:	a process of systematic and objective evaluation of all available information pertaining to a given hazard
Hazard identification:	the identification of biological, chemical and physical agents that may be present in a particular type of food or group of foods and are capable of causing adverse health effects.
Exposure assessment:	quantitative and/or qualitative evaluation of the likely intake of biological, chemical and physical agents via food as well as of the exposure to other sources
Hazard characterization:	quantitative and/or qualitative evaluation of the nature of the adverse effects associated with biological, chemical and physical agents that may be present in ingested food
Risk characterization:	the integration of hazard identification, exposure assessment and hazard characterization into a risk estimate of the likelihood and the severity of the adverse effects in a given population with attendant uncertainties.

Current microbiological risk assessments focus primarily on bacterial rather than viral hazards. For most foodborne bacteria, there have been standardized qualitative detection methods established. This is also increasingly the case for quantitative detection methods. Methods aimed at detecting or quantifying infectious viruses in foods are either more complex or not yet available. This implies that carrying out risk assessments for foodborne viruses is complicated by the limited data that are available. The current view is that undertaking a full quantitative risk assessment for foodborne viruses is not a realistic aim yet (FAO/WHO meeting report, 2008).

Early microbiological risk analysis concerned the safety of drinking water in which viruses were important target organisms. The primary focus was entero- and rotaviruses, for which culture methods and dose-response information were available (Gerba *et al.*, 1996; Haas *et al.*, 1993). Several studies on the availability of data for foodborne virus risk assessments are currently being performed. Thus, the Food Standards Agency (UK) has identified specific data gaps that need to be

addressed before a risk assessment can be undertaken for noroviruses in bivalve molluscs and fresh produce (FAO/WHO meeting report, 2008). A risk profile of viruses in foods has been prepared in New Zealand and the USA (FAO/WHO meeting report, 2008). However, the availability and the quality of the data not only vary from one country or continent to another, they also differ in terms of the target virus and the food products that are of concern.

STEPS

Hazard identification

Hazard identification in the present context consists in the identification of potential viruses in food products that are capable of causing adverse health effects. This step is usually based on a risk management issue. However, this issue should be well-defined, both as regards the targeted virus as well as the food product of interest. Indeed, it is not possible to carry out a risk assessment study for the entire range of potential foodborne viruses and food products. Hazard identification crucially relies on the availability of public health data and on an estimate of the sources and incidence of the hazard. Based on this information, which basically derives from surveillance data and epidemiological studies, it is possible to find out what are high risk products and processes. The incidence and severity of both HAV and rotavirus are well documented on a global basis, although not every country has the same degree of information or quality of data. An overview of potential foodborne viruses of interest and their priority level is shown in Table 1 of this report.

Hazard identification, which is based on the epidemiological record, focuses on HAV, rotavirus and NoVs, sapoviruses and HEV (Cliver *et al.*, 1997). Though the foodborne route of transmission for these viruses has been documented, it is less clear what proportion of viral diseases is attributable to food.

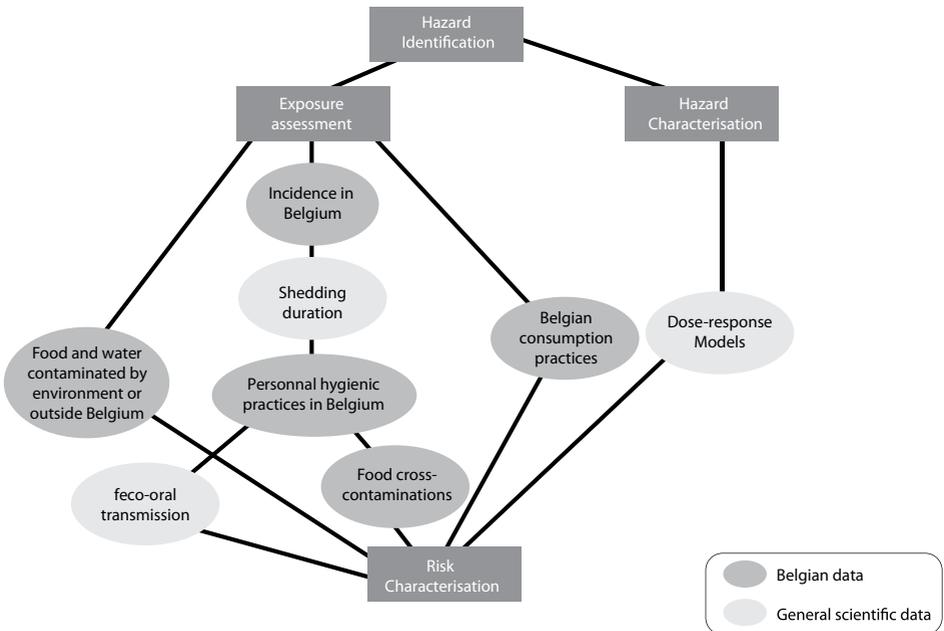
Exposure assessment

Once hazard identification is complete, it is possible to carry out an exposure assessment and a hazard characterization. Both steps can be performed simultaneously, though they require different expert approaches. Exposure assessment determines the likelihood of contaminated food being consumed. Ideally, it ascertains the distribution and the amount of pathogens of interest to which consumers may be exposed in a certain food product. It aims to quantify the exposure of consumers to the pathogens of interest via a given food product. In order to do so, it is necessary to know the probability of occurrence of viruses in a food item at the moment of consumption as well as the amount of viruses and their distribution. Several studies on HAV and noroviruses have already been conducted, using both molecular and cell culture methods to address this question.

Ideally, the effects at each stage of the production line and the transformation process should be assessed in order to build a model. The data provided in chapters IV and V are useful to feed the model and to study alternative scenarios at the stage of risk characterization. Figure 1 shows the main steps that need to be taken into account in order to carry out a model of risk assessment of foodborne viruses.

FIGURE 1.

Main steps that need to be taken into account in order to carry out a model of risk assessment of foodborne viruses in Belgium



There must be information available on the amount of food consumed and the frequency of consumption (Havelaar and Rutjes, 2008). Exposure assessment is one of the most complex and uncertain aspects of microbial risk assessment (Forsythe *et al.*, 2002). For instance, data on the amount of food products eaten during a meal are usually obtained from food consumption surveys and are similar to those from other microbiological risk assessments. However, some high risk food products, such as shellfish, may be consumed infrequently or by a limited proportion of the population. Hence, it may be more difficult to obtain specific data.

One major aspect that currently complicates the exposure assessment for foodborne viruses is the fact that there have been no standardized methods established for the qualitative (and quantitative) detection of viruses in food. Furthermore, as the distribution of foodborne viruses in the food supply is expected to be heterogeneous and non random, it is unlikely that there will be any virus detection methods applied to food on a routine basis. Indeed, the cost of performing predominantly negative tests would be huge (Cliver *et al.*, 1997). More often than not, there are no direct measurements available of the food contamination at the moment of consumption. Therefore, estimates are usually based on information obtained at earlier stages of the food chain (at harvest or at retail). For the purpose of carrying out this calculation, a batch of food is defined as being made up of a number of units. However, units (and size) can change along the food chain. Therefore, the further development of models and their adaptation for viruses in food remain a requirement (Havelaar and Rutjes, 2008).

As already discussed in chapter 2, the procedure for the detection of viruses in food can be divided into three steps: virus extraction from the food matrix, virus concentration and virus detection. More details on the impact of the detection method, which has been reviewed by Havelaar and Rutjes (2008), can be found in chapter 2. Special attention should be paid to the fact that most currently used methods determine the prevalence (the percentage of units contaminated by one or more infectious particles) on the basis of presence-absence tests. This falls short of the requirements for a quantitative risk assessment. Secondly, since virus recovery can vary significantly from one sample to another, the use of internal standards with every sample is recommended (see also European Committee for Standardization, CEN/TC275). However, this approach does not control virus extraction from the food item and might consequently overestimate virus recovery using this method. Third, virus recovery using cell culture methods, which are the only ones that are able to detect infectious viruses generally yields a lower outcome than virus recovery by means of molecular detection methods, which also detect non infectious particles. This indicates that virus recoveries based on molecular methods might be overestimated. As molecular methods detect nucleic acids and do not discriminate between viable infectious and non infectious virus particles, they are only of limited use to assess the virological safety of food. However, there are no reliable cell culture systems available for all foodborne viruses (Duizer *et al.*, 2004b). Therefore, the detection of viruses in foods currently focuses on the use of molecular techniques. The most frequently used molecular detection method is RT-PCR, which is based on the specific amplification of conserved regions of the virus genome. Though the technique is sensitive and specific, amplification can easily be inhibited by substances in the matrix. Therefore, the removal or inactivation of potential inhibitors remains a major determinant of effective virus detection.

In order to overcome the difficulties mentioned above, studies are being conducted in search of indicators that are able to predict the presence of pathogenic viruses in food. Although results are promising, the suitability of bacteriophages and human viruses as virus proxies in risk assessment models needs further research to evaluate whether a quantitative relationship can be established.

The study of Rose and Sobsey (1993) used and extrapolated data to characterize a highly infectious and a moderately infectious virus by means of a dose-response model based on rotavirus. It showed that the risk of a virus infection per single serving of shellfish was estimated to range between 1/100 if exposed to a moderately infectious virus and 5/10 if exposed to a highly infectious virus. As exposure assessments can provide greater insight into routes of transmission, and as there is already a certain amount of information available, conceptual models for this step can now be developed.

Hazard characterization

Hazard characterization provides an estimate of the nature, severity and duration of the adverse effects caused by the ingestion of a virus. This means that it is necessary to determine whether the severity of disease varies according to route of exposure (foodborne versus other routes) or whether it differs between healthy and more vulnerable individuals. One needs to consider whether differences in susceptibility should be addressed separately. Factors that are important for hazard characterization concern the nature of the target virus, the food content (e.g. fat and water content, consistency, pH) and the susceptibility of the host. Chapter 1 of this report reviews the information on the pathogenesis and clinical aspects of foodborne viruses.

An important step in hazard characterization is the dose-response relationship which is typical of the link between the ingested number of infectious virus particles and the probability of illness. Microbial dose-response models are based on basic assumptions that conceptualize the biological basis of host-pathogen interactions such as single-hit, independent action, and random distribution (WHO/FAO, 2003). Several models have been derived for the single-hit interaction, such as the exponential, the hypergeometric and the Beta-Poisson models (Zwietering and Havelaar, 2006).

Several experiments with viruses, including polio-, echo- and rotavirus, were performed in the 1950s (Zwietering and Havelaar, 2006). These studies clearly demonstrated the variability of the dose-response relationships depending on the properties of the virus, the host, and the matrix. The dose-response relationship for rotaviruses has been applied as a default to other human-pathogenic viruses in several studies. There are data available on NoV dose-response relations in human volunteers (Lindesmith *et al.* 2003; 2005). Yet these data cannot be extrapolated to infectious viruses, as there are none available on the infectivity of NoVs (Duizer *et al.*, 2004b). Moreover, a proportion of the population seems to be resistant to infection with some NoVs. This resistance is associated with the ABO histo-blood group type (Hutson *et al.*, 2002). There have been some human volunteer studies for HAV and rotaviruses using vaccine strains, but they have not been combined with food matrices or low virus doses (Teunis *et al.*, 1996). For emerging viruses such as hepatitis E virus, no studies are available on the basis of which a reliable dose-response could be determined. For those viruses that cause severe disease, the likelihood of obtaining

data is minimal. In conclusion, a consistent problem is the lack of any sort of dose-response data in which challenge has occurred in conjunction with the food matrices, as matrix effects have been shown to modify the dose-response relationship (Havelaar and Rutjes, 2008). Quantitative data from foodborne disease outbreaks could perhaps make it possible to propose powerful models for these viruses in the future.

Risk characterization

During risk characterization, the information from exposure assessment and dose-response models is combined into a risk estimate. Most current models assume that subsequent exposures are independent of earlier exposures, implying that there is no immunity effect. If this assumption should turn out to be incorrect, more complex models will need to be applied. Estimates of disease incidence can be extended to estimates of disease burden and costs to provide more information for decision making. In the Netherlands, the disease burden of noroviruses and rotavirus-associated gastroenteritis was 450 and 370 disability adjusted life years, respectively, whereas the total costs of illness were 23 million € and 22 million € per year, respectively (Havelaar and Rutjes, 2008).

Risk assessment

Current risk assessments of foodborne viruses are still predominantly focused on evaluating the safety of (irrigation) water, yet they seldom directly concern the contamination level of food products (Hamilton *et al.*, 2006; Masago *et al.*, 2006; Petterson *et al.*, 2001; Rose and Sobsey, 1993; Stine *et al.*, 2005). The current lack of quantitative data makes it difficult to take a quantitative approach to risk assessment, yet this doesn't rule out the use of risk assessments for particular foodborne viruses. When there are no data available, it is possible to resort to assumptions, although the latter must be unambiguously identified as such and this must be clearly stated. Conceptual models can be developed as well. An important contribution of risk assessment could lie in the identification of data requirements and the prioritising of further experimental and observational studies. As the infectious dose of many of these viruses is still largely unknown, qualitative models of risk assessment could provide the decision makers with some preliminary interesting insights, in particular concerning the control measurements that need to be implemented in the food chain.

Current risk assessment models typically focus on one single exposure event and do not take into account secondary transmission or the effect of previous exposures. The evaluation of secondary transmission may also be of critical importance, both with respect to specific settings as well as the general population. Furthermore, those who have recovered from illness are generally assumed to have developed protective immunity. There have been studies on the impact of secondary transmission and immunity on the environmental transmission of viruses, but their findings are not commonly applied yet (Eisenberg *et al.*, 1996; 2004).

Furthermore, determining the ratio of infectious to non infectious virus particles is a very uncertain undertaking that is subject to high variability. Yet this is a general problem in assessing the health risks associated with the detection of viruses in food products. It is also desirable to have better dose-response information. One should take into consideration the assumption that foodborne viruses have a high infectivity. Finally, as stated by Havelaar and Rutjes (2008), it is also necessary to take into account person-to-person transmission and immunity by incorporating dynamic models in the further development of risk assessments.

Chapter VII – RECOMMENDATIONS FOR BELGIUM

General considerations

- Foodborne viruses are assigned a level of priority that is based on their association with foodborne transmission (Table 1). **Noroviruses and HAV** are categorised as **level 1 viruses**, as they are the most common causes of foodborne viral outbreaks. Sapovirus, HEV and rotavirus are assigned to level 2. Aichivirus, TBEV, astrovirus, adenovirus and enterovirus are all level 3 viruses. Indeed, there are few reports in which foodborne outbreaks are attributed to these viruses.
- There is no certainty over the **infectious dose of foodborne viruses**. On the one hand, it is estimated to be extremely low: approximately **10 to 100 virus particles**. On the other, foodborne viruses are often shed at extremely high titres that can reach up to **10⁹ virus particles/g faeces**.
- According to assessments carried out with sensitive techniques, norovirus and HAV can be **shed for up to several weeks** after a symptomatic or asymptomatic infection. There should be special hygiene measures recommended for individuals working in health care or food workers manually preparing or handling foods. Additional precautions should be taken with respect to individuals who have been found to excrete these viruses.
- Foodborne viruses are mainly spread by the faecal-oral route. **Person-to-person** transmission is significant. There have been cases reported in which the viruses had been transmitted by contaminated **food, drinking water or recreational water**. At present, it is not possible to determine which fraction of the total incidence of human illness due to viral pathogens is attributable to foods, and to what extent this concerns particular foods.
- **Fresh produce** can be considered as a **high risk food**. It can be contaminated at the pre-harvest level by contact with faecally polluted irrigation water, organic-based fertilizer, or food pickers (harvest). There is no certainty over the extent to which each of these potential routes of transmission is involved. Limited data are available on the presence of viruses in different types of water that are used in the primary plant production in Belgium. Furthermore, the contamination risk factors that result from fresh produce being in contact with polluted irrigation water are not well known.
- **Manually treated food intended to be consumed without further heating, such as catered food, can pose a high risk** for viral contamination. Because of the low infectious dose and the large amount of virus shed, infected food workers should be removed from work until at least the end of the acute illness. Additional hygiene measures need to be implemented once they have returned from illness.

- **High risk foods** also include **shellfish**, as these animals are filter-feeders: viruses are filtered out of the surrounding polluted water. These viruses are retained and can even be concentrated in the digestive tissue of the bivalve molluscan shellfish (oysters, mussels, cockles, clams). If contaminated shellfish are consumed raw or only slightly cooked (just until the shells are open), this will hold a risk for viral infection.
- A full risk assessment of the major viral pathogens, NoV and HAV, in the high risk foods mentioned above is not available at the moment and will be difficult to perform. Such an assessment requires a better understanding of the transmission routes, prevalence, persistence and infectious particle titres of these viruses in the food supply chain. In addition, there are currently insufficient quantitative data available.
- The mandatory microbiological limits (EC n°854/2004 and n°2073/2005) that need to be observed whilst checking live bivalve shellfish are based on the level of bacterial indicators (**E. coli and faecal coliforms**), **not on the presence of viruses**. Additional viral indicators that point to the presence of human pathogenic viruses are therefore required.
- Unlike bacteria, viruses cannot grow outside their host. As a result, they cannot be grown in culture media. Furthermore, most **foodborne viruses cannot be cultivated** in cell culture **in the laboratory** or show fastidious growth. As a consequence, they are detected by means of **molecular detection assays**. **Reverse transcriptase (RT)-PCR** is the pre-eminent technique for detecting foodborne viruses. In order to obtain reliable test results, it is necessary to carry out **adequate controls of the molecular detection assays, including an** internal amplification control to check PCR inhibition and a process control to check sample processing.
- With no culturing methods available, virus extraction requires adequate **methods** to prepare small volume samples from the food for (RT-)PCR, even when there are only low numbers of viruses present. It is not possible to apply any **horizontal viral detection methods**. It seems necessary to categorize foods according to their composition (e.g. foods of the fatty type, of the watery type). **Harmonisation** and categorization is still ongoing in Europe (CEN), as well as worldwide (US, Canada). There is a need for extensive ring-trials to select robust, simple and reliable viral extraction methods.
- As viral detection relies on molecular detection, it targets the **viral genome**. The molecular detection assay will reveal whether or not there are any viral genome copies present. A positive result indicates that there has been viral contamination. However, the fact that viral genomic copies have been detected by means of (RT-)PCR does not necessarily mean that there are infectious viral particles present. Given this state of affairs, novel detection methodologies are required which are able to **distinguish between infectious and non infectious viral particles**.

- Good agricultural practices (GAP), good manufacturing practices (GMP) and good food hygiene practices (GHP) are of major importance to avoid the viral contamination of food products. The frequent occurrence of viral food borne outbreaks shows that these “good practices” are not always met in the food supply chain. Typical shortcomings concern the effectiveness itself of the preventive measures and poor procedure compliance (e.g. poor cleaning practices, unhygienic behaviour). Procedure compliance may be influenced by guideline and procedure awareness and knowledge, but also by the persistence of existing habits and attitudes. The risk of contamination can be reduced by vaccinating food-handlers. Such vaccines are already available for HAV and poliomyelitis, but not for NoV.
- **Food preservation methods** that are based on the inhibition and inactivation of microbial growth need to be assessed **in order to determine their effectiveness** in reducing/eliminating **foodborne viruses**. There are insufficient data on the stability of viruses that are subjected to food processing technologies.
- Outbreaks in elderly homes and cruise ships have been traced down to **contaminated surfaces** as well as nursing staff and food-handler hands, which is indicative of the stability of foodborne viruses. More data are required on the effectiveness of cleaning and disinfecting agents (biocides).
- **The investigation of foodborne outbreaks** needs to be improved. This will require sufficient resources to **enhance the network** between the Reference laboratory of foodborne outbreaks, which analyses the foods, and the epidemiological unit, which collects epidemiological information. This will reduce the under-reporting of viral foodborne outbreaks in Belgium.
- The analysis of clinical samples for virus detection should be encouraged and alternative sources of financing should be found. This will in turn lead to reduced underreporting, thus improving the estimate of the burden of foodborne viral disease to society.
- The zoonotic properties of **foodborne viruses** as well as the presence of **animal reservoirs** are still **being investigated**. At present, there is no evidence that production or companion animals play a part in the transmission chain of noroviruses. However, HEV is present in pigs, which makes it necessary to clearly determine the significance of this reservoir. The presence of sapoviruses and aichiviruses in production animals is a point of interest that also requires special attention.
- Noroviruses are not known to the general public in Belgium. Also many doctors, health workers in semi-closed institutions such as hospitals, nursing homes and day care centres are not aware of the existence of this virus. As NoVs are **highly contagious**, they can easily be spread. NoVs normally cause mild gastroenteritis, but they can also lead to **severe disease** in sensitive groups such as **young children, the elderly and immunocompromised individuals**.

- **It is strongly recommended to deliver information** on foodborne viruses (NoV, HAV) to medical doctors, to those working in health care or with sensitive groups and to those in charge of food safety management systems in the food chain. It is also advised to provide specific and appropriate training to food-handlers.

Considerations for future research

- Fresh produce is to be looked upon as high risk food. The routes of virus transmission onto fresh produce are not clear. Still, water is generally acknowledged to be a potential route of transmission. Limited data are available in Belgium on the presence of viruses in different types of water used in primary plant production. It is necessary to shed light on the link between viral contamination and the presence of faecal indicators and bacterial pathogens.
- Furthermore, the contamination risk factors that result from fresh produce being in contact with polluted irrigation water are not well known. In addition, there is no information available about the attachment, adherence and/or internalization potential of foodborne viruses in the tissue of fruits and vegetables, nor on their survival in the ecological niche formed by the crop.
- More data are needed on the stability of foodborne viruses that are subjected to food processing technologies. Viruses are suggested to be more stable in the environment than bacteria. This requires further examination. Inactivation rates should be defined on the basis of various model viruses.
- The resistance of viruses should be examined under various physical and chemical conditions that mimic those that are reached during the production process.
- One should assess the effectiveness of disinfectant biocides against the relevant foodborne viruses.
- The presence of foodborne viruses or related viruses in domestic animals calls for a better understanding of their potential zoonotic transmission.
- Data on the molecular epidemiology of human and animal noroviruses and HEV (zoonotic risk and animal reservoir) are needed for future intervention and for the prevention programme, both of which are based on their role as a potential zoonotic agent or on the presence of an animal reservoir.
- There is a need for prospective studies that investigate the virus-host interaction of viruses which will potentially emerge, such as HEV or Aichivirus.
- It is necessary to develop novel methodologies that can distinguish between infectious and non-infectious foodborne viruses. Improving risk assessment involves quantifying these viruses as well as assessing the doses that are infectious for humans more accurately. This will help to determine the risk for public health whenever viruses are detected in foods, water or in the environment by means of molecular techniques such as RT-PCR.

Chapter VIII - CONCLUSIONS

Epidemiological data clearly show that food can act as an effective vehicle for the transmission of viruses, even though only a fraction of the cases are reported to the national surveillance system. In fact, a lot of countries do not have any surveillance at all.

There are three different ways in which viruses can be transmitted via food, i.e. food-handlers (through ready-to-eat food), bivalve molluscs and water (on fresh products). Viruses do not replicate in food and food products, which means that their quantity will never exceed the initial viral load. The fact that the viral load in the analysed food sample is usually low also explains why it is so difficult to detect viral contamination. It follows from these considerations that molluscs are to be looked upon as a special food product. Indeed, they are filter-feeders and therefore concentrate virus particles in their digestive tract.

The most important viruses in terms of the number of cases and the severity of illness are noroviruses and hepatitis A virus. Other significant foodborne viruses are hepatitis E virus (HEV), human rotaviruses and human sapoviruses. HEV has been included in this category because it is now being reported in Belgium in humans and pigs. Most of these viruses have a faecal-oral route of transmission. Other viruses are of minor importance, i.e. astroviruses, adenoviruses types 40 and 41, aichivirus, enteroviruses and tick-borne encephalitis virus (TBEV). This report did not go into the details of some emerging zoonotic viruses that are believed to be transmitted via food, such as H5N1 avian influenza A virus, Nipah virus and rabies virus.

Although the most common viral pathogens induce a fairly mild, self-limiting illness, their high incidence is indicative of their potential to cause large international foodborne viral epidemics. Nevertheless, there is also a risk of more dangerous illnesses, such as hepatitis, being transmitted through food.

Good epidemiosurveillance will not only help to identify any changes in the epidemiological profile of viruses like HEV, Aichivirus or TBEV, it will especially contribute towards detecting their emergence or at least noticing an increase in their incidence. Indeed, these viruses have a high potential of emergence in northern Europe.

The genetic proximity of some animal and human viruses raises questions about zoonotic transmission and animal reservoirs. In order to improve our understanding of the way in which viruses like noroviruses and HEV are transmitted, it is necessary to sequence the circulating animal and human strains. There should also be collaborative research on public and animal health.

Chapter IX - REFERENCES

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Chapter X – COMPOSITION OF THE WORKING GROUP

All experts joined the working group *in a private capacity*. The names of the experts of the Superior Health Council (CSS-HGR) are indicated with an asterisk*.

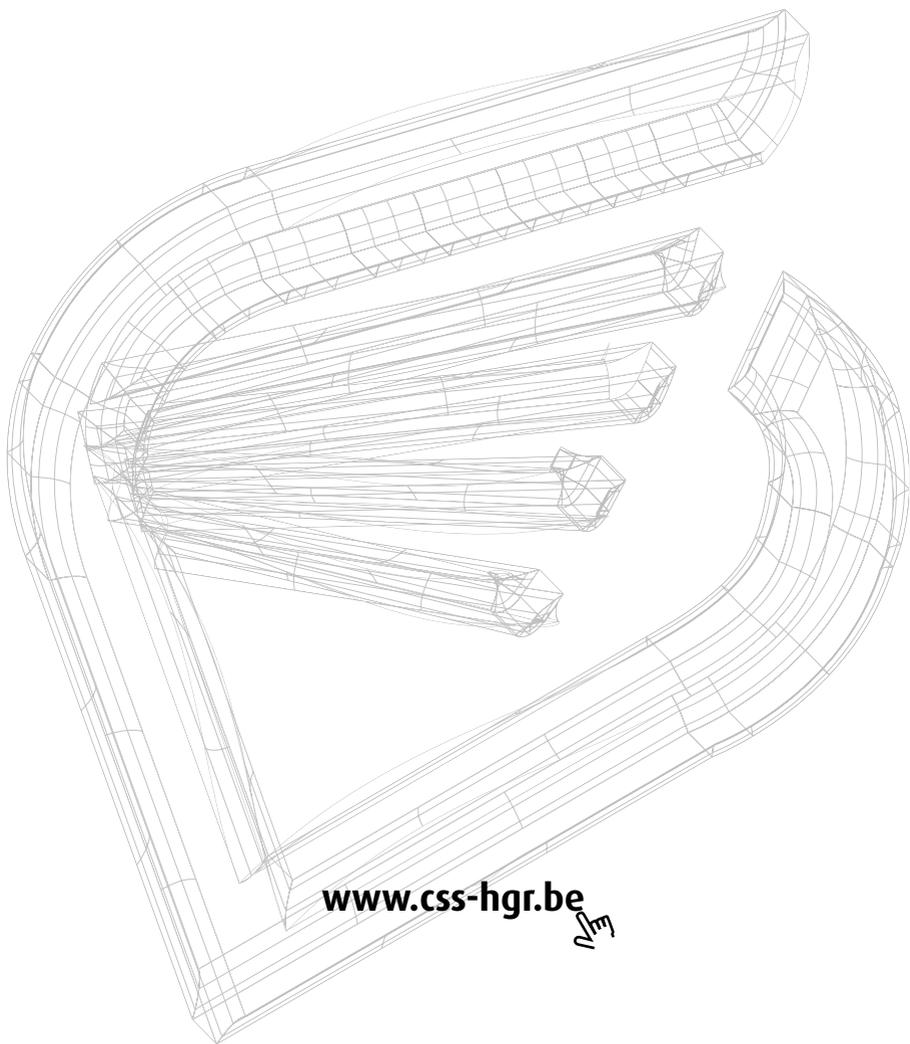
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The working group was chaired by **Etienne THIRY**, the scientific secretary was **Jean-Jacques DUBOIS**.

This group wishes to thank **Ms Christina ESPERT** (ULg) for her helpful contribution in finalising this report and **Ms Evelyn HANTSON** (CSS-HGR) for her very useful improvements to the English version of this scientific report.

This text was also approved by the “*Food Microbiology WorkingGroup*” of the Superior Health Council.



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