codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 13 (b) **CX/FH 05/37/14**

February 2005

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON FOOD HYGIENE

Thirty-Seventh Session

Buenos Aires, Argentina, March 14 – 19, 2005

DISCUSSION PAPER ON THE VIRUSES IN FOOD

Prepared by the Netherlands

BACKGROUND

Since the preparation of the document CX/FH 99/11, "Discussion Paper on Viruses in Food" (see Appendix to this document) for the 32nd Session of the Codex Committee on Food Hygiene (CCFH), significant progress has been made in understanding of viruses that may be transmitted through the food chain. Some of the most important areas of progress are highlighted in this document. This is not an exhaustive review, but should be seen as a sampling of the different fields, focused primarily on the description of the public health problem.

EPIDEMIOLOGY

Studies of community acquired infectious intestinal disease have been done in The Netherlands and in the UK and have demonstrated that viral infections account for a large proportion of community-acquired gastro-enteritis, especially the noroviruses. For The Netherlands (population 16 million) it is estimated that >500.000 cases of norovirus illness occur in the community during the study period (1999). Many smaller surveys in limited populations have confirmed the high burden of illness due to noroviruses.

In a risk factor analysis, using data collected with questionnaires during the community-based study, it was estimated that 12-17% of the norovirus infections in The Netherlands are likely to be food-related. This could not be specified further, to define risks for specific food items, but gives estimates for the incidence of food-borne norovirus disease in the same range as for *Salmonella* and *Campylobacte.*

Besides the high impact of noroviruses as a cause of "sporadic" cases in the community, they are probably best known for their propensity to cause outbreaks in institutional settings. There are a large number of outbreak surveys published since 1999, which describe noroviruses as causes of food- or waterborne illness, caused by for example, contaminated shellfish, raspberries.

While these outbreak descriptions clearly indicate the potential for food-related norovirus infections, little work has been done to map the proportion of all outbreaks attributable to specific modes of transmission. However, outbreak surveillance systems that were developed in Australia, the US and Europe are beginning to map the proportion of reported outbreaks that are thought to be caused by viruses, and again, viruses are

increasingly identified as the etiologic agent. A problem in comparability of data is that there is very little standardization in outbreak reporting and virus detection. Therefore, international comparison of data is a challenging task.

A study across Europe found between 0 and 21 outbreaks of norovirus illness per million population in the different countries. These differences most likely result from differences in the setup and / or quality of the surveillance. Approximately 10 % of these outbreaks were reported as likely to be linked to food- or waterborne transmission, although the level of evidence was often descriptive. Only a small proportion of all reported outbreaks had data recorded on the suspected vehicle for infection. Of these, 12% was attributed to oyster consumption, the rest to a mix of other food- items. It is difficult to separate food- and waterborne transmission. Several case reports illustrate that water-borne transmission of noroviruses occurs, and probably is underreported. A survey that found viral genes in a high proportion of bottled mineral waters sparked a lot of discussion but could not be confirmed by others. The published reports on waterborne illness however illustrate that they may be difficult to detect through routine surveillance without targeted studies.

INDIRECT IMPACT OF FOOD-ASSOCIATED DISEASE

The amplification of food-borne infections through person-to-person transmission is an issue that needs further consideration. The initial outbreaks will occur in people who ate e.g. oysters, but secondary and tertiary waves of infection may occur, which then are recognized as person-to-person outbreaks. While technically speaking they are indeed person-to-person outbreaks, one could argue if these too should be attributed to food. This is exemplified by the following: In the winter of 2000/2001 several outbreaks of norovirus illness developed in 3 countries around New Year, associated with imported shellfish. The viruses clearly stood out, because they were of an unusual type that had not been observed in most surveys prior to that date. Tracking of this virus learned that over 200 outbreaks occurred in 7 countries, following this initial introduction.

VIRAL GENETICS

In addition, in the above example, it was shown that this was a highly unusual virus lineage, because it consisted of 4 different recombinant genomes. Recombination can only happen if two viruses infect the same cell at the same time, and mix their genetic material to form essentially a novel virus. Since in the oyster outbreaks that have been examined, often more than one virus is found, it can be postulated that consumption of multiply contaminated oysters constitutes an extra risk for generation of novel norovirus strains. This is essentially the same mechanism as the scenario that leads to the generation of a new pandemic influenza virus. So far, the virulence of the novel strains has not been different, but this is a very uncontrolled situation.

In 2002, Europe and the US were swept by a wave of outbreaks of illness with another new variant norovirus, which somehow popped up around January and caused a large excess over the “usual” number of outbreaks observed in previous years. Reports published later showed that these variants had already circulated in the community for some months, leading to unusual numbers of hospitalization which were only recognized retrospectively and through a targeted study. Another “epidemic wave” occurred in the winter of 2004/2005. Both epidemics occurred over a vast geographic region. The mechanisms of evolution of these viruses remains unclear, but given the ample evidence for food- and waterborne transmission, food is likely to play a role in the dissemination of such novel variants.

PROGRESS IN VIRUS DETECTION IN FOOD

Since 1999, a lot of work has gone into the development of methods for virus detection in food. Here, the situation differs for shellfish and for other food items. Methods for virus detection in shellfish have been used with some success, and the EU has initiated a network of reference laboratories. This network is working on harmonization of virus detection methods across Europe. Most progress has been made at the

technical level, but data on the application of these methods for monitoring purposes remain patchy. The question on how to implement such methods in surveillance is far from being answered.

Virus detection in other foods is far less advanced. There are some case reports in which food-borne outbreaks were confirmed by the identification of the outbreak strain in a food item, but at present these are the published exceptions. Given the nature of virus contamination, a fundamental question is whether food monitoring in a manner similar to bacterial contaminants will ever be reliable. Therefore, data about levels of contamination of food will be difficult to obtain. Therefore, a question is if fundamental modifications in the risk analysis approach are needed when applying this to viruses.

PROGRESS IN UNDERSTANDING HOST FACTORS

A breakthrough in the field has been the discovery that clear differences in susceptibility have been found between persons with different blood groups and other genetic markers. This is explained by the observation that norovirus particles bind to carbohydrates that are part of the histo-bloodgroup antigens. Further research has shown that the binding properties differ between different genetic variants, thus providing very different patterns of host susceptibility. Put in simpler terms: not every norovirus variant has the same impact. Studies in humans have shown that a particular variant, the genogroup II.4 noroviruses seems to be predominant across the world, especially as a cause of outbreaks in institutional settings such as nursing homes, hospitals). Interestingly, in oysters, viruses belonging to a different genogroup (GGI) are found more frequently. It is unclear why this is. A very intriguing observation was that binding (and therefore accumulation) of noroviruses in oysters appears to be related to binding to similar carbohydrates, and that the presence of such carbohydrates differs between strains of oysters. Therefore, some types of oysters may be more effective in accumulating noroviruses than others.

ZOONOTIC TRANSMISSION

The potential for zoonotic transmission has been raised in the 1999 discussion document. With the increasing availability of data from surveillance in humans, no viruses have been identified that could be matched with strains found in animals. Therefore, it is not likely that noroviruses are directly zoonotic in a manner similar to *Salmonella*. However, the viruses found in animals and humans are genetically related, and the question remains if under certain circumstances genetic mixing could occur, leading to the generation of novel viruses. Systematic data from high-risk regions for such events, namely regions in which humans and animals live in close contact under low hygienic conditions, are not available.

EMERGING VIRAL DISEASES

For hepatitis E, there is now solid proof that food-borne infections do occur, and that HEV is endemic in regions that were previously considered free from these viruses. The exact modes of transmission are still under study. Very similar viruses have been identified in pigs and in humans in several regions of the world (Japan, US, Europe).

During the SARS epidemic increasing evidence became available for the potential for fecal-oral spread of SARS coronavirus. Similarly, with the increasing threat of avian influenza virus for humans, issues of the potential for food-borne introduction and spread of these viruses have been raised. While there is no evidence for these viruses that food-borne transmission played a role in their transmission to humans, this has highlighted the importance of targeted control measures for the event of a new virus introduction into the food chain. The data available for norovirus could serve as a template for the identification of knowledge gaps and shortcomings in the current control of food-borne viral infections.

The weaknesses in the current level of control of transmission of viruses via the food-chain are increasingly visible in the literature and are becoming common knowledge due to the increased public interest following the epidemics of norovirus outbreaks in 2002 and 2004. Many of the popular media have run items about

noroviruses and how they are transmitted. This raises issues about the vulnerability of the food chain for intentional contamination with viruses.

CONCLUSIONS

- *Epidemiological studies since 1999 have shown that viruses and especially noroviruses are the most commonly diagnosed causes of community acquired gastro-enteritis, including food-related illness. Currently the best lower estimates are that approximately 10-15% of community cases and outbreaks can be attributed directly to food-borne transmission of noroviruses*
- *Systematic outbreak surveys are rare, and at present provide sufficient level of proof for common-source events in a small proportion of outbreaks only. When available, data consistently support a role for food-borne transmission of noroviruses.*
- *The recent example of SARS and the threat of avian influenza and bioterrorism have raised important questions about the potential for food-borne transmission of such high threat pathogens.*
- *Food-borne outbreaks have occurred in which people are exposed simultaneously to mixtures of viruses. These viruses may exchange genes to form novel variants. From the virological perspective, this situation is highly undesirable because of the unpredictable nature of the outcome of such mixing (recombination) events. Both SARS and pandemic influenza are examples of viruses that can lead to epidemics due to abrupt genetic changes.*
- *At present, there is little evidence for zoonotic transmission of noroviruses, although genetically related viruses have been found in several animal species. The question if genetic mixing between animal and human viruses could lead to a new variant pathogenic for humans remains unanswered and can not be excluded at this stage.*
- *Progress in the field of virus detection in food is slow and fraught with technical complexities. It is unlikely that routine data on monitoring of food items for viruses will become available in the near future, with the possible exception of data for shellfish.*

RECOMMENDATIONS

A Codex Task Force is proposed which will develop, in its role as Risk Manager, a Risk Profile to be used to define a Risk Assessment policy and subsequently assign a (noro)Virus in Food Risk Assessment.

The task force should focus principally on reviewing existing documents and available relevant data appropriate for assessing and managing the risks connected with (noro)Virus in Food. This activities should result in a Risk Profile enabling to define purpose and scope of a (noro)Virus in Food Risk Assessment to be executed by JEMRA.

On the basis of the Risk Profile purpose and scope of a (noro)Virus in Food Risk Assessment should be defined.

When results of the (noro)Virus in Food Risk Assessment become available the Task Force should develop Risk Assessment based Guidance to reduce the risks of (noro)Virus in Food.

The first Task Force meeting should convene in 2005.

The (noro) Virus in Food Risk Profile should be available and the Risk Assessment policy defined early in 2006, so that a JEMRA (noro) Virus in Food Risk Assessment can be assigned.

The results of the Risk Assessment should become available in the course of 2007.

The (noro)Virus in Food Task Force will use the results to develop Codex draft (noro)Virus in Food Risk Management Guidance to be finalized in 2008 or 2009. The draft risk management guideline will be available in 2008/9, which would allow for adoption and endorsement, respectively, in 2009.

APPENDIX

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Agenda Item 11 **CX/FH 99/11**
October 1999

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON FOOD HYGIENE

Thirty-second Session

Washington, DC, U.S.A., November 29-December 4, 1999

Discussion Paper on Viruses in Food

(Prepared by the Netherlands with the assistance of Finland, Germany, Italy, USA)

BACKGROUND

At the 31st Session of the Codex Committee on Food Hygiene, the Delegation of the Netherlands brought forward for the Committee's review a scientific paper on caliciviruses[1] and proposed that CCFH should consider food safety hazards associated with viruses with a view to developing recommendations for their control.

The Committee considered the paper and recognized that, awaiting the formation of an expert advisory body on microbiological risk assessment, it might be useful to review matters related to foodborne viral diseases, in the framework of a discussion paper to clarify issues.

The Committee agreed that the Delegation of the Netherlands would prepare a discussion paper on the subject in cooperation with several other interested countries[2].

Representatives of the Drafting Group (chaired by the Netherlands with assistence of Finland, Germany, Italy and the United States of America) met and developed this *Discussion Paper on Foodborne Viral Infections*.

This paper provides a comprehensive review of foodborne and waterborne viral gastroenteritis with a focus on caliciviruses and hepatitis. The document also reviews high risk foodstuffs, virus detection in food and water and the current status of prevention and disinfection.

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[1] CRD 23, *Foodborne infections by Norwalk-like caliciviruses (syn. Small round structured viruses, SRSV)*. M. Koopmans.

[2] ALINORM 99/13A, paragraphs 116 – 118.

1. GENERAL INTRODUCTION

1.1. INTRODUCTION

Foodborne- and waterborne viral infections are increasingly recognized as causes of illness in humans. Reasons for this increase are most likely the improved diagnostic assays that have enhanced detection of some virus groups, and changes in food processing and consumption patterns that lead to the worldwide availability of high risk food (32). As a result, vast outbreaks may occur due to contamination of food by a single foodhandler or at a single source, as has been documented for the Norwalk group of viruses on several occasions.

Numerous viruses can be found in the human intestinal track (Table 1). The food- and waterborne viruses can be divided into three disease categories:

i) Viruses that cause gastro-enteritis (astrovirus, rotavirus, adenovirus types 40 and 41, and the two genera of enteric caliciviruses, the small-round-structured-viruses or “Norwalk-like viruses" (NLV), and typical caliciviruses or “Sapporo-like viruses”, (SLV).

ii) Faecal-orally transmitted hepatitis viruses: hepatitis A virus (HAV), hepatitis E virus (HEV)

iii) Viruses which cause other illness, e.g. enteroviruses.

In addition, several viruses are listed that also replicate in the intestinal tract, but are not implicated in foodborne transmission, or whose role is unknown.

Viruses, unlike bacteria, are strict intracellular parasites and can not replicate in food or water. Therefore, viral contamination of food will not increase during processing, and may actually decrease. This implies that viral infection via contaminated food depends on the following:

1. Viral stability
2. Amounts of virus shed / degree of viral contamination
3. Processing of food or water
4. Likelihood of infection after ingestion of 1 virus particle
5. Susceptibility of the host

Most food- or water-borne viruses are relatively resistant to heat, disinfection and pH changes. It is no coincidence that most virus groups implicated in outbreaks are small, non-enveloped particles, rather than large, fragile, enveloped viruses. Problems in the detection of viral contamination of food or water are that -generally- the contaminated products will look, smell, and taste normal, and that (molecular) diagnostic methods for most of these viruses are not routinely available in food microbiology laboratories. In this paper, the major viral causes of foodborne infections will be reviewed. We have focussed on those viruses that are most commonly transmitted by food, namely caliciviruses and hepatitis A virus.

Table 1. Enteric viruses grouped according to the associated clinical syndrome.

Gastroenteritis	*Possibly gastroenteritis*
Rotavirus group A, B, C	Picobirnavirus
Adenovirus types 40,41	Torovirus
Astrovirus serotypes 1-8	Coronavirus
Norwalk-like caliciviruses	Cytomegalovirus
Sapporo-like caliciviruses	HIV
	Parvo-like viruses, SRFV (Wollan, Ditchling)
Hepatitis	**OTHER**
Hepatitis A virus	Enteroviruses: polio 1-3 coxsackie A 1-22, 24 coxsackie B 1-6 echo 1-9, 11-27, 29-34 entero 68-71
Hepatitis E virus	Parvovirus?

1.2. INTERNATIONAL DEVELOPMENTS

The importance of foodborne transmission of viruses is increasingly recognized, and the World Health Organization has signaled an upward trend in their incidence. It is also understood that the burden of infection is grossly underestimated by routine surveillance. The aging population (with increasing numbers of people at risk for complications of enteric infections) and the globalization of infectious diseases due to rapid international travel and (food) trade add to the notion that the burden of illness is likely to increase in the years to come. This is reflected by the attention of national and international organizations: food-borne infections have been ranked as the Number One public health concern in the European Union, by a concerted effort of 14 public health institutes. In several countries (The Netherlands, UK, France, Finland) the Ministers of Public Health and Agriculture have asked Public Health Councils or expert groups for advise on the burden of illness and possible prevention of food-borne infections, given their high and increasing incidence (Advisory Committee on microbiological safety of food, 1998). In addition, ministries in The Netherlands, UK, and France have commissioned large-scale epidemiological studies to monitor trends in the incidence of gastroenteritis in different populations.

In Finland, as a result of the work of an expert group, an enhanced surveillance program (including virological examination) was initiated in 1997 and led to considerably more registered cases of food- and waterborne epidemics. In the United States, vice president Al Gore has launched a multimillion dollar initiative to control the upward trend in foodborne infections.

(**Fout! Bladwijzer niet gedefinieerd.**.)

In this initiative, the need for better diagnostic methods for - among others- foodborne viral infections has been stressed. The European Parliament and Council has agreed to develop a surveillance network for

epidemiological surveillance and control of infectious diseases in the European Union (Directive 2119/98/EG).

1.3. COST OF ILLNESS.

The cost of illness due to viral food borne infections is not known exactly, but it is likely to be high. In the USA, some 9,000 deaths and 81 million illnesses each year have been attributed to consumption of contaminated food (**Fout! Bladwijzer niet gedefinieerd.**.). For just the few foodborne pathogens for which cost estimates have been made, medical charges and lost productivity already cost society $5-6 billion annually in the USA (81). The estimated total costs of Salmonellosis are $1.2-1.5 billion. For comparison: it is becoming clear from epidemiological studies that caliciviruses alone may be as frequent causes of illness and even deaths as *Salmonella* (14*).* Although viral enteric infections generally cause mild illness, costs can be high due to their frequent occurrence and high transmissibility. Outbreaks in institutions can create major logistic problems, when, as is typical of gastroenteritis outbreaks, 30-40% of staff is affected at a time when the level of patient care needed is highest (94, 95). Frequently, such institutions can only curb the problem by closing wards down to new admissions. In addition, there are studies that indicate that viral enteric infections cause deaths in the elderly, deaths that are largely preventable (14, 18, 44, 56). The contamination of foods and the subsequent illness may also have serious economic consequences, as evidenced by two recent relatively small outbreaks in Denmark and in Sweden following consumption of shellfish and raspberries, respectively: although in these outbreaks the cause of illness was not actually proven, in both cases a temporary marketing ban was announced.

In the USA, some 60,000 cases of hepatitis A are reported annually, of which an estimated 7.3% cases were foodborne or waterborne Outbreaks of hepatitis A are common in crowded situations such as institutions, schools, prisons, and in military forces. The percentage of adults with immunity increases with age, but the age at which most infections occur is increasing in Europe. The increased number of susceptible individuals allows common source epidemics to evolve rapidly, and the likelihood of such epidemics is increasing (67).

2. FOOD- AND WATERBORNE VIRAL GASTROENTERITIS, WITH A FOCUS ON CALICIVIRUSES

2.1. INTRODUCTION

In most epidemiologic studies of food- and waterborne viruses, samples have been screened for viruses by tissue culture isolation techniques or by electron microscopy (EM). Some enteric viruses, however, can not be grown in tissue culture, and EM is not a very sensitive method for the detection of these viruses. Simple diagnostic tests, such as enzyme-linked immunosorbent assays (ELISA), have only been reported for group A rotavirus and adenovirus in clinical specimens. No similar assays exist for testing food samples. As a result of these limitations, foodborne viral gastroenteritis is usually not diagnosed.

In the absence of virus detection assays, a tentative diagnosis of viral gastroenteritis can be made based on epidemiological criteria described by Kaplan et al. (41). Characteristic features are: acute onset after a 24-36 hour incubation period, vomiting and/or diarrhea lasting a few days, a high attack rate (average 45%), and a high number of secondary cases (31, 41). Using this approach, an estimated 32-42% of foodborne enteric infections in the USA are caused by viruses. Outbreaks of gastroenteritis may be caused by rotaviruses, astroviruses, adenoviruses (type 40 and 41), and the human enteric caliciviruses. The human caliciviruses are assigned to two genera: “Norwalk-like viruses” (NLV), also known as small-round-structured-viruses or SRSV, and “Sapporo-like viruses” (SLV), also known as typical caliciviruses (38, 39, 59). The NLVs cause illness in people of all age groups, whereas the SLV predominantly cause illness in children (40).

The relative importance of the different viruses as causes of food- and water-borne infections is not exactly known, but clearly caliciviruses are the main cause of viral outbreaks (41), and their incidence reportedly has been increasing in recent years (62, 99). This “emergence” of caliciviruses as the main foodborne virus most likely is not a true increase in incidence, but rather an increased awareness combined with improved diagnostic

assays. Large water- and food-borne outbreaks of group B and C rotavirus have been described in China and Japan, respectively (31). The remainder of this chapter will focus on NLV, unless otherwise indicated.

2.2. CLINICAL SYMPTOMS

Following a 1-3 day incubation period, infected persons may develop (low grade) fever and vomiting, diarrhea, and headache as prominent symptoms. The symptoms usually subside within two to three days, although the course of illness may be protracted in the elderly. Deaths associated with NLV outbreaks have been reported. The average attack rate is high (typically 45% or more). Virus is shed via stools and vomit, starting during the incubation period, and lasting up to 10 days, and possibly longer (36, 78). NLV infections are highly contagious, resulting in a high rate of transmission to contacts. Note: since contaminated foods may contain multiple agents, mixtures of symptoms may occur.

2.3. EPIDEMIOLOGY

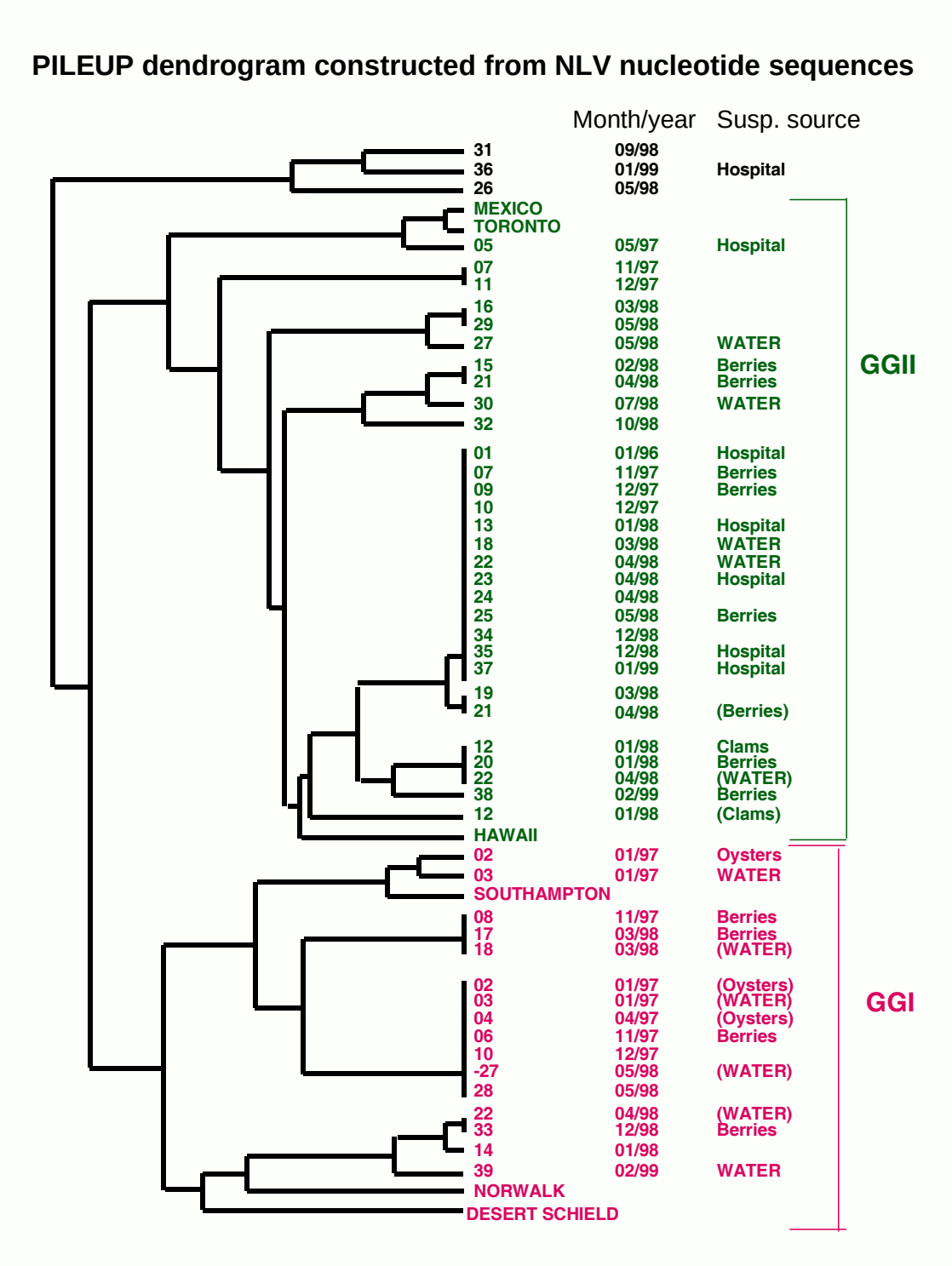
Following the development of molecular detection methods, it has become clear that NLV infections are among the most important causes of gastroenteritis in adults and often occur as outbreaks which may be foodborne (Table 2). In The Netherlands, approximately 80% of outbreaks of gastroenteritis that are reported to municipal health services are caused by NLVs (95). More than half of these outbreaks occurs in nursing homes. The proportion of foodborne outbreaks was 17% from 1994-1999, with 70% of these attributed to NLV (45). This most likely is an underestimate as foodborne outbreaks are usually reported through the regional food inspection services, rather than municipal health services. In a survey of all outbreaks of infectious intestinal disease in England and Wales between 1992 and 1994, 27% of outbreaks were caused by NLV (32% of outbreaks were due to *Salmonella* spp). NLV were the cause of 6% of foodborne outbreaks. Since outbreak specimens were mostly examined by electron microscopy, the actual numer of NLV outbreaks is likely to be higher (14). In the US, 86 of 90 (96%) of outbreaks of nonbacterial acute gastroenteritis reported to CDC between January 1997 and June 1998 were caused by NLV infection. Of those outbreaks for which a mode of transmission was reported, 24 of 51 (47%) were considered foodborne (15). Similarly, In Finland, hospital outbreaks (mostly on geriatric wards) are almost exclusively caused by NLV, but there is serious underreporting. In Finland, 56% of the epidemics reported as food-borne, and from which stool samples (and foodstuff, in some instances) have been submitted for virological screening, were NLV-positive (65). Of water epidemics 12/15 have been NLV-positive. Since 1998 15 (rasp) berry-related epidemics occurred, which has resulted in a ban on the use of unheated raspberries in all catering and other large-scale kitchens (79). Since then, some (rasp)berry-associated outbreaks occurred, in cases where the ban was neglected. Most of these outbreaks were linked to imported (rasp)berries. From molecular typing it was shown that many different lineages of NLV could be found, which illustrates that contamination of these foods was not linked to a single common source (Figure 1).

Table 2. Summary table of outbreak studies, in which samples were tested for NLV.

Country	*Study*	*Proportion foodborne*	*% NLV (of foodborne)*	*Method*	*Reference*
US	Foodborne outbreaks	100	32-42	Epidemiology	Kaplan, 1982
UK	All outbreaks	50	6	EM	Djuretic, 1996
US	Non-bacterial outbreaks	47	96	PCR	Fankhauser, 1998
NL	All outbreaks	17	70	PCR	Vinjé, 1996, 1997; Koopmans, 1999
Finland	Food-and waterborne	100	56	PCR	Maunula, 1999

In addition to outbreaks, recent publications suggest that caliciviruses are among the most common causes of sporadic gastroenteritis (45, 75, 97). In The Netherlands, 5% of patients who visit their physician for gastroenteritis were infected with NLV (compared with 4% for *Salmonella*), as well as 17% of persons in a sentinel population who developed diarrhea in the winter months (1, 33, 45). People from all age groups were affected, with a slightly higher incidence in very young children.

Figure 1: Phylogenetic tree showing NLV lineages that were found in outbreaks of gastroenteritis in Finland (identified by number), including 12 outbreaks in which raspberries were implicated as the most likely source of infection. GG = genogroup. Mexico, Toronto, Hawaii, Southampton, Norwalk and Desert shield virus are reference strains of NLV.



2.4. RISK GROUPS

Outbreaks of NLV-gastroenteritis (not only foodborne) are common in institutions such as nursing homes and hospitals. The high attack rate in both residents and personnel of such institutions often leads to major understaffing problems during outbreaks. Sporadic cases of viral gastroenteritis also occur frequently in these settings. The risk factors for these infections are currently under investigation in the UK and in The Netherlands. According to Gerba et al (21) the group of individuals who would be at the greatest risk of serious illness and mortality from water- and food-borne enteric microorganisms includes young children, the elderly, pregnant women, and the immunocompromized. This segment of the population currently represents almost 20% of the population (in the United States) and is expected to increase significantly by the beginning of the next century, due to increases in life-span and the number of immunocompromized individuals. Worldwide, diarrheal diseases account for millions of deaths annually, mostly in developing countries. In developed countries, mortality due to diarrhea is low, but does occur in young children (7, 11, 76) and in the elderly (>50%; 18, 21, 56). While specific mortality data on NLV are not available, given the high incidence of calicivirus infections in the elderly, it is likely that deaths resulting from calicivirus infection do occur.

2.5. MOLECULAR EPIDEMIOLOGY

Within the NLV genus, a great diversity of virus types exist, based on analysis of the genomic sequence and antigenic characterization (57, 58, 73). To date, 15 distinct genotypes have been recognized, but as more strains are characterized, this number is likely to increase. It is well established that many different types of NLV cocirculate in the general population, causing sporadic cases and outbreaks. Typically, strain sequences are (almost) identical within outbreaks, and different when specimens from different outbreaks are analyzed. Thus, when identical sequences are found in different patients or different clusters of illness, a common source for the infection should be suspected. Conversely, finding different sequences in people with a supposedly common source infection suggests independent contamination, unless there is an association with sewage-contaminated water: in epidemics due to sewage contamination, often more than one strain is encountered (47, 65, 88).

Occasionally, epidemics occur in which the majority of outbreaks are caused by a single genetic type (e.g. in The Netherlands in 1996; 95). These epidemics may be widespread and even global (74). The mechanisms behind emergence of epidemic types are unknown. Hypotheses include large-scale foodborne transmission of a single strain, and spillover from a possibly non-human reservoir. Recently, NLVs were found in pigs in Japan and in cattle in the UK (12, 60, 89).

2.6. IMMUNITY

Little is known about immunity to NLV infections. From experimental infections in volunteers it is known that infected persons may develop immunity, but only for a short period, and limited to the infecting genotype or highly related strains (29, 73). Volunteers with antibodies to the infecting genotype have a higher risk of illness and a steeper dose-response curve (26, 72). It is unclear what this means. The lack of broadly reactive, long-lived immunity to natural infection suggests that development of a protective vaccine may be problematic.

2.7. TRANSMISSION

NLVs are transmitted by direct person-to-person contact or indirectly via contaminated water, food or environmental surfaces. Clearly, person-to-person transmission is by far the most common route of infection. However, many foodborne NLV outbreaks have been described, often resulting from contamination by an infected foodhandler (15, 20, 45, 65, 94, 95). It is important to note that contamination may occur not only at the end of the food distribution chain, but at almost any step from farm to table. Infected foodhandlers may transmit infectious viruses during the incubation period and after recovery from illness (20, 61, 76). In addition, several waterborne outbreaks of NLV have been described, both directly (e.g. consumption of tainted water) or indirectly (e.g. via washed fruits, by swimming or canoeing in

recreational waters)(6, 9, 27, 46, 47). There are some anecdotal indications for aerogenic transmission of NLV, but it is unclear if this route is of major importance.

Besides person-to-person transmission via food vehicles, zoonotic transmission has been reported for some enteric viruses. Based on similarities between viral isolates, transmission of rotaviruses from monkey, cat, dog, horse and cattle to humans is possible. There are no reports that have addressed this issue, but at the same time there is little evidence for zoonotic transmission as a major source of foodborne rotavirus infections (23, 98).

Until recently, the NLV were considered to be pathogens with humans as the sole host. Recently, however, NLV were found in healthy pigs in Japan and in historic calf stool specimens from the UK and from Germany (12, 60, 89). The calf viruses, named Newbury agent and Jena virus are pathogenic for young calves. The two bovine enteric caliciviruses and the pig enteric calicivirus are genetically distinct from human strains, but cluster within the NLV genus. In a pilot study in The Netherlands, pooled stool samples from calves, fattening pigs, and adult cows were tested for the presence of NLV. Thirty three (45%) of the calf herds tested positive for a NLV strains belonging to the Newbury genotype, and one pig herd was found positive for a virus which was very similar to the pig calicivirus from Japan (45). These findings raise important questions on the host range of the NLVs. At this stage it is unclear if the animal NLVs form genetically distinct stable lineages, or are in fact part of a common pool of viruses co-circulating between animals and humans.

2.8. DIAGNOSIS IN HUMANS

In stool specimens, viruses can be detected by electron microscopy (all viruses), immunoassays (rotavirus group A, adenovirus types 40/41, astrovirus), and molecular methods (all viruses described). The detection limit varies for different methods with EM as the least sensitive method (detection limit around 10^6 particles per ml), and reverse-transcriptase polymerase chain reaction (RT-PCR) amplification as the most sensitive method (detection limit 10-100 particles per ml)(94). Thus, the successfull detection of viruses in stool specimens depends on the methods used and maximum virus titers shed (rotavirus 10^{10} per ml, astrovirus 10^8 per ml, NLV 10^7 per ml). Since the titre of virus shed decreases during illness, it is important that stool specimens are collected in the first days following onset of symptoms, and are stored at 4°C.

NLV or SLV infections historically have been diagnosed by visualization of virus particles by electronmicroscopy. At present, several broadly reactive RT-PCR assays are available, that can be used both for detection and for genotyping. (28, 73, 94). NLV have been divided into two genogroups (Figure 1) and tentatively into 15 genotypes.

3. HEPATITIS VIRUSES

3.1. INTRODUCTION

The viruses which cause hepatitis can be divided in enterically transmitted viruses (hepatitis A virus, hepatitis E virus), and bloodborne hepatitis viruses (Hepatitis B, C, D, G) . For food- or waterborne transmission, only the enterically transmitted viruses are relevant. Hepatitis A virus (HAV) is a virus in the family *Picornaviridae*, to which also the enteroviruses belong (including poliovirus). Hepatitis E virus shows some resemblance with viruses from the family *Caliciviridae* (to which the NLV belong), but has is not (yet) been included in a virus family because of some unique characteristics.

Hepatitis E has only relatively recently been established as a cause of hepatitis, when large waterborne outbreaks occurred in India and Pakistan. Foodborne outbreaks of hepatitis E virus have not been documented. The virus is endemic over a wide geographic area, primarily in countries with inadequate sanitation where hepatitis A is endemic as well (South-East Asia, Indian subcontinent, Africa), but not as widespread as HAV. In industrialized countries hepatitis E infections are rare, and are usually travel-related (48, 91, 92). There are some indications that Hepatitis E infections may be transmitted from an animal reservoir (70, 71). Hepatitis E outbreaks can be distinguished based on the higher attack rate of clinically evident disease in persons 15-40 years of age compared with other groups, higher overall case fatality rates (0.5-3%), and the unusually high death toll in pregnant women (15-20%). In younger age groups, the majority of hepatitis E infections may present without jaundice, unlike clinically apparent hepatitis A infection (67). Since HEV can cause illness

with high mortality in pregnant women, a study of foodborne virus transmission in our opinion should include HEV.

The remainder of this chapter will deal with hepatitis A solely, since most information is available on this pathogen, and it is an established foodborne pathogen. This does not imply that hepatitis E may not be important, but information on this pathogen and its role in foodborne illness is sparce.

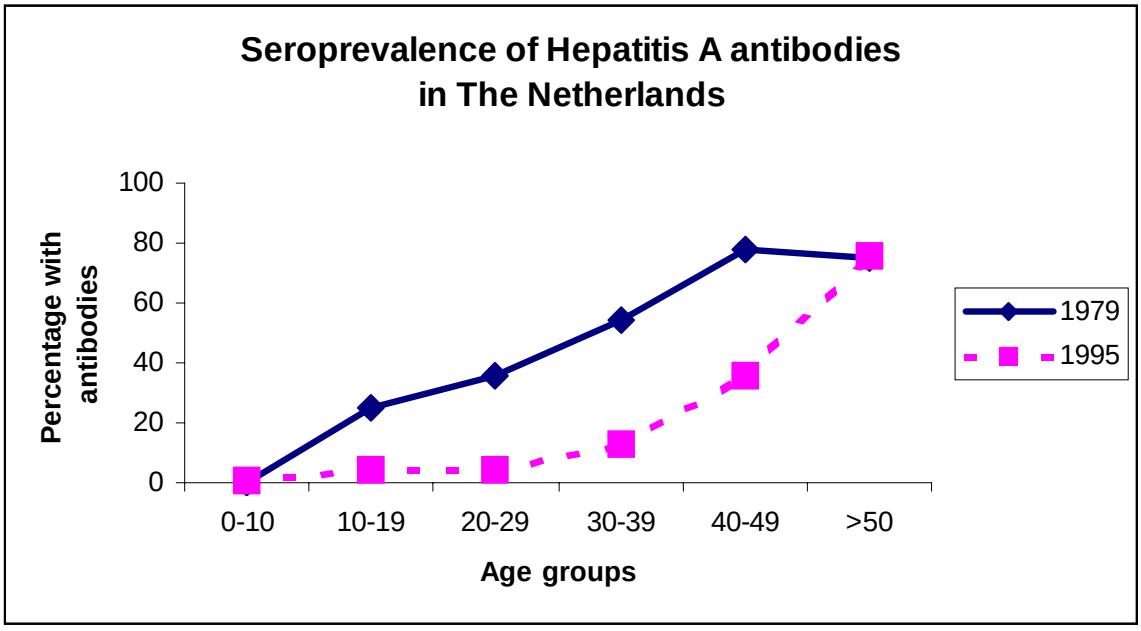
3.2. CLINICAL SYMPTOMS

After a 2-7 week incubation period, HAV infection will result in non-specific symptoms like fever, headache, fatigue, nausea and vomiting, followed by signs of hepatitis 1-2 weeks later. Virus shedding typically continues untill 1 week after onset of jaundice in adults and 1-2 weeks in children, although prolonged intermittent shedding may occur for up to 90 days in patients with clinical relapses. Relapses have been reported in 1.5-18.5% of persons (85). Young infants may shed virus up to 5 months after infection (84).

3.3. EPIDEMIOLOGY

Hepatitis A has been endemic worldwide, but the incidence has decreased dramatically in many regions by sanitary measures only. Ironically, after an initial decrease this has led to an increase in the number of clinical cases: when hepatitis A infections are less common, less people will develop immunity at an early age, and the population at risk will increase (Figure 2). As a result, infections may occur more commonly in older age groups (e.g. while travelling to endemic areas). In young children, who become infected in areas where the virus is endemic, most infections (>95%) do not lead to symptoms and will go undetected; in contrast, in adults, HAV infection may result in rather serious illness in 70-80% of persons, with a case fatality rate of up to 3% (67). In England and Wales, the annual notification rate of HAV infection has risen fourfold between 1987 and 1991 from 3.6 to 14.6 per 100000 population (63). For this same reason, the outbreakpotential of HAV has increased (67). The number of notifications declined considerably in the early sixties and stabilized in the seventies at 5-7 notifications per 100,000 inhabitants. In Italy, data collected from a surveillance system for type specific acute viral hepatitis (SEIEVA) showed that the incidence of HAV declined from 10/100.000 in 1985 to 2/100.000 during the period 1987-1990, while an increase was observed after 1991. The highest attack rate was observed in the 15-24 year age group (68).

Figure 2: Seroprevalence of antibodies to hepatitis A virus in The Netherlands in different age groups in 1979 and 1995. Data adapted from references 17 and 66.



In a case control study of hepatitis A in England, the factors associated with increased risk of hepatitis A included travel (odds ratio [OR] 19.8; 95% confidence interval [CI] 4.9-80.6), a household contact with hepatitis A (OR 13.5; CI 6.5-28.0), sharing a household with a child aged 3 to 10 years (OR 1.57; CI 2.2), consumption of bivalve molluscs (OR 1.7; CI 1.2-2.4), and consumption of untreated water (OR 1.85; CI 1.1-3.0)(63). Additional risk factors are attendance or employment at a day care center, exposure to infected food or water during an outbreak, homosexual activity, and injecting drug use. No known risk factors are identified in many cases.

From the studies in Italy, again shellfish consumption (OR = 2.6; CI = 2.4-2.9), travel to endemic areas for people residing in northern and central Italy (OR = 5.4; CI = 4.6-6.2) and having a child in day-care (OR = 1.2; CI = 1.03-1.4), were all independently associated with an increased risk of HAV infection (68). Shellfish consumption was the most frequently reported source of infection over the period considered. Several food- and waterborne borne outbreaks of hepatitis A related to consumption of contaminated food have been described (10, 13, 35, 55, 66). In 1996 and 1997, a large HAV epidemic occurred in Southern Italy, Puglia region, with 11.000 notifications especially among young adults. The main risk factor in this epidemic outbreak was consumption of mussels (64). A large hepatitis A epidemic occurred in Finland among drug abusers (around 300 cases) due to contaminated amphetamine. In outbreak situations, up to 20% of cases are due to secondary transmission.

3.4. RISK GROUPS

Since the case-fatality rate of hepatitis A infection increases with age, risks are higher for older age-groups, provided they have not encountered hepatitis A virus throughout their life. The decreasing seroprevalence of hepatitis A combined with the ageing of the population results in an increased likelihood of outbreaks and a more serious course of illness (16). Persons with hepatitis C infection and possibly those with chronic hepatitis B, are at increased risk for fulminant hepatitis following superinfection with hepatitis A (42, 93).

3.5. MOLECULAR EPIDEMIOLOGY

Molecular detection and - typing assays have been developed for Hepatitis A virus (8, 82, 83). They have been evaluated for use with stool specimens from patients with hepatitis (90). Seven genotypes of hepatitis A virus have been recognized, 4 of which occur in humans. The other three genotypes have been found in captive old world monkeys (54). Patterns of endemic transmission can be differentiated from situations in which infections are imported due to travel by sequence analysis of hepatitis A strains from patients. This genomic diversity can and has been used to pinpoint the source of foodborne outbreaks (35, 66), and waterborne outbreaks (13).

3.6. IMMUNITY

A single hepatitis A infection appears to induce lifelong immunity. Only one serotype of hepatitis A has been found, but genetically distinct lineages are found in different geographic regions. There are inactivated hepatitis A vaccines that are highly immunogenic and confer a high level of protection to HAV infection. There are indications that early post-exposure vaccination may also be protective (2, 3).

3.7. TRANSMISSION

Hepatitis A virus is readily transmitted from person to person. In addition, food- and waterborne transmission have been documented for hepatitis A virus. The risk of contracting infection through viral contamination of fresh fruits which are imported from many regions in the world is increasingly recognized (35). During the short viremic phase, bloodborne transmission is possible (2).

Hepatitis A virus can survive for 12 weeks to 10 months in water, and as a result infection can occur by ingestion of a variety of shellfish from sewage-contaminated areas. Waterborne outbreaks have been reported, both in association with drinking fecally-contaminated water and with swimming in contaminated swimming pools and lakes (67).

3.8. DIAGNOSIS IN HUMANS

Diagnosis of hepatitis A infection is made by detection of virus-specific IgM antibodies in serum. In addition, virus (up to 10^9 particles per ml) can be detected in stool samples by molecular methods between three and five weeks after infection with hepatitis A virus, usually starting well before the onset of clinical symptoms. This latency period is a problem for infection prevention. In addition, relapses may occur in up to 18% of people, who may then shed virus for several months (85) Detection of hepatitis A virus in shellfish and water has been described (section 4.2).

4. GENERAL ASPECTS

4.1. HIGH RISK FOODSTUFFS

Shellfish are notorious as a source of foodborne viral infections, because they actively concentrate virus from contaminated water (43). Depuration, a practice that may reduce bacterial contamination, is not effective in reducing viral contamination. Several other foods, however, have also been implicated as vehicles of transmission (desserts, fruits, vegetables, salads, sandwiches): the bottomline message is that any food that has been handled manually and not (sufficiently) heated subsequently is a possible source of infection (14). It is important to note, however, that contamination may occur not only at the end of the food chain, but at almost every step in the path from farm to table.

4.2. VIRUS DETECTION IN FOOD AND WATER

Although diagnostic methods have been developed for the detection of virus or viral RNA in food and water, they have not found their way to routine laboratories in most parts of the world (4, 5, 19, 22, 24, 25, 37, 49-53). Most studies of virus detection in food have focussed on shellfish, for which several groups have developed slightly different protocols, and comparative studies are needed to determine which assays should be recommended. It remains unclear what the predictive value is of a negative test. This information is needed before screening of such specimens can be done to monitor contamination.

A special problem is that caliciviruses can not be grown in tissue culture, and hepatitis A viruses only with moderate success. As a result, data on the correlation between the presence of viral genes (as tested by RT-PCR) and viable virus are lacking. For outbreak diagnosis, the current approach is the screening of stool specimens from cases and controls, combined with an epidemiologic investigation to assess food-specific attack rates. Foods with a significant odds-ratio may then be examined by molecular methods, although no information is available about the sensitivity of these methods for outbreak diagnosis. Quality control of food and water on the basis of the detection of indicator organisms for fecal contamination has proven to be an unreliable predictor for viral contamination.

For shellfish, both screening of growing waters or of shellfish could be done, but the relative sensitivities of these approaches need to be evaluated. When NLVs are detected in food, typing assays can be used to establish transmission routes, and to support or refute epidemiological links with cases.

4.3. PREVENTION AND DESINFECTION

Increasing the awarenes of all foodhandlers about transmission of enteric viruses is needed, with special emphasis on the risk of “silent” transmission by asymptomatically infected persons and those continuing to shed virus following resolution of symptoms. While it may be unclear what proportion of foodborne infections can be attributed to workers in different parts of the food chain, it is important that viruses become part of science-based Hazard Analysis and Critical Control Point (HACCP) systems to identify risks and to help identify gaps in knowledge (e.g. Table 3). At present, insufficient data are available to determine which steps are going to be critical for all foods. Preventive measures differ for the different transmission routes.

i) Shellfish: for shellfish, strict control of the quality of growing waters can prevent contamination of shellfish. This includes control of waste disposal by commercial and recreational boats. Guidelines specifically aimed at reduction of viral contamination are needed, as it has become clear that the current indicators for water and shellfish quality are insufficient as predictors of viral contamination.

ii) Food items contaminated by infected foodhandlers: personal hygiene is most important in preventing foodborne viral infection, and includes frequent handwashing and wearing gloves. This should apply for all points in the food chain where foodstuffs are handled manually. The ID50 (dose resulting in infection of 50% of exposed individuals) of NLV appears to be extremely low (72). As a result, even with strict sanitary measures, infection may not always be prevented. Foodborne outbreaks have occurred due to contaminated food sources that passed all microbiological assays. A common sense guideline is to remove people with symptoms consistent with viral gastro-enteritis from the production chain untill at least two days after remission of the symptoms. A practical problem with this guideline is that an unknown proportion of viral infections will be subclinical, and that -even in the incubation period- infected persons may shed sufficient amounts of virus to cause food-contamination (61). The kinetics of viral shedding have only been studied in a few infected volunteers, and may not reflect the real life situation when people may have been infected with a low dose of infectious virus. Given the highly infectious nature of NLV, and the documented risk of virus transmission to food during the incubation period, it is envisioned that guidelines should be developed that consider the occurrence of gastroenteritis in contacts (e.g. children) of people working in critical points in the food chain. This should be based on data on the kinetics of viral shedding following natural infection.

The globalization of the food market has hampered the implementation of control measures to assure safe food. It is not clear whether routine monitoring of food specimens for viral contamination will be feasible. However, for prevention of foodborne transmission, it is also essential that food items are not grown or washed in fecally contaminated water.

Documented outbreaks of foodborne infections could be reported faster using, for example, the “rapid alert system for food” of the European Union or the US Foodnet and would be much more informative if typing information of virus strains would be included.

As for the other enteric viruses, personal hygiene is most important in preventing foodborne viral hepatitis infection. Problems include the long incubation period, and the facts that infected people shed the highest levels of infectious virus before the onset of illness and that infections may not lead to clinical symptoms. As a result, again, several foodborne outbreaks have been described in which an infected foodhandler was the source of infection (30, 86). In addition, transmission of infection has occurred through fresh fruits grown in areas where the fruits were sprayed with fecally-contaminated water. This implies that products for human consumption should only be grown with high quality water. A vaccine is available for hepatitis A, and contacts can be treated with the administration of immunoglobulin. The Advisory Committee on Immunization Practices (ACIP), USA, recommends HAV vaccination for 1) persons traveling to countries that have high or intermediate endemicity; 2) children in communities that have high rates of HAV infection; 3) men who have sex with men; 4) Illegal-drug users; 5) persons who have occupational risk for HAV; 6) persons who have chronic liver disease; 7) persons who have clotting-factor disorders; 8) other groups, possibly food handlers (2). Whether HAV vaccination is feasible for preventing foodborne transmission for specific countries or regions depends on many local factors (e.g. level of endemicity, hygienic conditions) and needs to be evaluated for these specific situations, based on HACCP analysis.

NLVs can survive outside the host, are resistant to common disinfectants and extreme pH fluctuations, and are highly infectious. As a result, transmission of virus via fomites is likely.

It is important to note that contamination can be widespread after vomiting, due to airosol formation and subsequent transport of virus particles by air. The effect of desinfectants on NLV infectivity has hardly been studied, due to the lack of a trissue culture system or animal model. From experiments with adult volunteers in the 80s it has been suggested that Norwalk virus (one of the prototypes NLV) is resistant to low pH (2.7), ether extraction, and heat treatment (30 minutes at 60°C). The virus reportedly is quite resistant to chlorine as the virus remains infectious after 30 minutes in the presence of 0.5-1 mg free chlorine per liter. At higher concentrations, the virus is inactivated (>2 mg per liter free chlorine**;** 40). These findings have to be interpreted with caution, as data from recent dose-response studies makes it clear that very high doses of virus were used in earlier volunteer challenge experiments. Therefore, reduction of infectivity due to various treatments may not have been detected.

Based on semiquantitative detection by using PCR-units, drinking water treatment processes using coagulation-flocculation-sedimentation, filtration, and disinfection with free chlorine, monochloramine, ozone, clorine dioxide or UV irradiation all reduce the amount of Norwalk virus more than 4 log steps (87).

Hepatitis A virus supposedly is resistant to low pH (up to pH1), and is resistant to heat as it survives 1 hour at 60º (54)

Table 3: Stages in knowledge about foodborne infectious pathogens: NLV (adapted for viruses from a presentation by R. Tauxe: Emerging foodborne diseases: an evolving public health challenge. 17th International Conference of the International Committee on Food Microbiology and Hygiene, 1999*)*

What is the disease?	Gastroenteritis
What is the microbial pathogen?	NLV
How can it be easily identified in people?	By molecular detection assays
How can it be identified in food?	?? Some assays available, but information lacking on predictive value of a negative test.
How common is the infection?	Number one cause of outbreaks of nonbacterial foodborne gastroenteritis
Which foods are the sources?	1. Shellfish. 2. Any food that has been handled manually and not heated afterwards
How did the pathogen get into the food?	1. Infected foodhandlers, anywhere from farm to table. 2. Irrigation or washing with fecally contaminated water. 3. Zoonotic transmission unclear.
How can it be treated?	Only symptomatic treatment
How can it be prevented?	Strict hygiene
Does the prevention strategy work?	?

5. LEGISLATION, RULES AND REGULATIONS

Statutory sanitary control for shellfish is called for by Council Directive 91/492/EC, which states that shellfish for the market must contain ≤ 230 *Escherichia* coli or in 100 g of shellfish flesh. However, this bacteriological parameter is inadequate for the control of viral contamination since lack of correlation was shown between the presence of viruses and coliform bacteria (25, 96); in fact HAV has been detected in mussels that otherwise meet bacteriological standards. The investigation and surveillance of zoonosis will be required also under the European Parliament and Council Directive on surveillance of zoonosis (DGVI, document VI/99EN-Rev.1a).

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

- Up untill now detailed knowledge on virus in food was not compiled; with this "Discussion Paper on Viruses in Food" a start for assembling information has been made. The data in the paper demonstrate that viruses in food can be an important source of foodborne illnesses.
- Many relevant advices are given in this Discussion Paper. The suggestions speak to the critical need for research, studies and evaluations to more clearly delineate needs such as those related to:
  - Determining the common routes of virus transmission, including foodborne infection
  - Improving surveillance for illness and tools for the molecular tracing of viruses throughout the food chain
  - Developing new and improved methods for the detection and typing of foodborne viruses, and using these methods for food screening
  - Developing surveillance plans to determine which are high risk foods in connection to virus comtamination
  - Determining the mechanism of emergence of epidemic strains, including the link with porcine calicivirus infections
  - Evaluate if public campaigns directed at prevention of viral foodborne infections are likely to be successful
  - Evaluate the use of sludge waste and wastewater for irrigation for risks of viral contamination of food
  - Consider the incoporation of foodborne viruses in food safety programmes
- When designing a hygiene control program, it is important to take the above advices into consideration, especially so in environments where viruses from either human, animal or environmental could cause significant contamination of food products.
- Existing Hygienic measures which are intended to control bacterial food infections, might help prevent foodborne virus infection, however this measures are not validated to this effect in most cases. That means that virus infections can not be excluded, when "traditional" hygiene measures are properly applied.
- At this stage, as documented in this paper, the available science concerning foodborne virus infection is not yet sufficient to establish adequate control measures in the format of a typical Codex Guideline. This might be possible in a later phase, when the state of the art provides more practicable control measures.

6.2 RECOMMENDATION

- In case of review of this “Discussion Paper on Viruses in Food”, it is recommended to make the present limited knowledge about the discussed topic accessible to as many people as possible. Not only to further expand the knowledge, but also to contribute to the prevention of virus infections through food, where achievable. A suitable format to communicate the available knowledge to the global community could be a Codex paper “Control measures for Foodborne Viruses”, comparable to the paper as presently prepared for Listeria monocytoges.

7. REFERENCES

1. Abbink F., Duynhoven Y., Wit M.A.S. de, Koopmans M.P.G., Leeuwen W.J. van, Kortbeek L.M. A population cohort study with a nested case-control study: a study design to estimate the incidence and aetiology of gastroenteritis in the Netherlands. Abstracts 4th World Congress on Foodborne infections and intoxications, Berlin, june 1998:171:P-A032.
2. ACIP. Prevention of hepatitis A through active or passive immunization. Recommendations of the advisory committee on immunization practices. MMWR 1996;45/RR-15:1-30.
3. Anon. Hepatitis A vaccine: evidence of efficacy after exposure.Commun Dis Rep CDR Wkly. 1999;9(16):139, 142.
4. Atmar, R.L., Metcalf, T., Neill, F., and Estes, M. Detection of enteric viruses in oysters by using PCR. Appl Environm Microbiol 1993;59:631-635.
5. Atmar RL, Neill FH, Romalde JL, LeGuyader F, Woodley CM, Metcalf TG and Estes M. Detection of Norwalk virus and hepatitis A Virus in shellfish tissues with the PCR. Appl. Environ. Microbiol., 1995;61:3014-3018.
6. Beller M., Ellis A., Lee S.H., Drebot M.A., Jenkerson S.A., Funk E., Sobsey M.D., Simmons O.D. 3rd, Monroe S.S., Ando T., Noel J., Petric M., Middaugh J.P., Spika J.S. Outbreak of viral gastroenteritis due to a contaminated well. International consequences. JAMA. 199720;278:563-8.
7. Bern C., Lew J., McFeeley P., Ing D., Ing R.T., Glass R.I. Diarrheal deaths in children living in New Mexico: toward a strategy of preventive interventions. J Pediatr. 1993;122:920-2.
8. Birkenmeyer LG, Mushahwar IK. Detection of hepatitis A, B and D virus by the polymerase chain reaction. J Virol Methods. 1994;49(2):101-12.
9. Brugha R., Vipond I.B., Evans M.R., Sandifer Q.D., Roberts R.J., Salmon R.L., Caul E.O., Mukerjee A.K. A community outbreak of food-borne small round-structured virus gastroenteritis caused by a contaminated water supply. Epidemiol. Infect. 1999;122:145-54.
10. CDC. From the Centers for Disease Control and Prevention. Hepatitis A associated with consumption of frozen strawberries--Michigan, March 1997. JAMA. 1997;277(16):1271
11. Crowley D.S., Ryan M.J., Wall P.G. Gastroenteritis in children under 5 years of age in England and Wales. Commun. Dis. Rep. CDR Rev. 1997;7:R82-6
12. Dastjerdi AM, Green J, Gallimore CI, Brown DW, Bridger JC. The bovine Newbury agent-2 is genetically more closely related to human SRSVs than to animal caliciviruses. Virology. 1999;254:1-5.
13. De Serres G., Cromeans T.L., Levesque B., Brassard N., Barthe C., Dionne M., Prud'homme H., Paradis D., Shapiro C.N., Nainan O.V., Margolis H.S. Molecular confirmation of hepatitis A virus from well water: epidemiology and public health implications. J Infect Dis. 1999;179(1):37-43.
14. Djuretic, T., Wall, P.G., Ryan, M., Evans, H.S., Adak, G.K., and Cowden, J.M. General outbreaks of infectious intestinal disease in England and Wales 1992 to 1994. CDR Review 1996;6:R57-63.
15. Fankhauser RL, Noel JS, Monroe SS, Ando TA, Glass RI. Molecular epidemiology of small round structured viruses in outbreaks of gastroenteritis in the United States. J Infect Dis 1998;178:1571-8.
16. Forbes A., Williams R.Changing epidemiology and clinical aspects of hepatitis A.Br Med Bull. 1990;46(2):303-18.

17. Frösner, G., Papaevangelou, G., Butler, R., Iwarson, S., Lindholm, A., Courouce-Pauty, A., Haas, H., and Deinhardt, F. Antibody against hepatitis A in seven european countries. Am. J. Epidemiol. 1979;110:63-69.

18. Gangarosa, R.E., Glass, R.I., Lew, J.F., and Boring, J.R. Hospitalizations involving gastroenteritis in the United States, 1985: the special burden of the disease among the elderly. Am. J. Epi. 1992;135:281-290.

19. Gajardo R, Dmez JM, Jofre J, Bosch A. Adsorption-elution with negatively and positively-charged glass powder for the concentration of hepatitis A virus from water. J of Virol Meth 1991;31:345-352.

20. Gaulin C.D., Ramsay D., Cardinal P., D'Halevyn M.A. Epidemic of gastroenteritis of viral origin associated with eating imported raspberries. Can J Public Health. 1999;90:37-40. French.

21. Gerba CP, Rose JB, Haas CN.Sensitive populations: who is at the greatest risk? Int. J. Food Microbiol. 1996;30(1-2):113-23.

22. Gilgen, M, German, D., Lüthy, J. and Hübner, P. Three-step isolation method for sensitive detection of enterovirus, rotavirus, hepatitis A virus and small round structured viruses in water samples. Int J Food Microbiol.1997; 37:189 – 199.

23. Glass, R.I., Gentsch, J., en Smith, J.C. Rotavirus vaccines: success by reassortment? Science 1994;265:1389-1391.

24. Gouvea, V., Santos, N., Carmo Timenetsky, M., and Estes, M. Identification of Norwalk virus in artificially seeded shellfish and selected foods. J. Virol. Meth. 1994;48:177-187.

25. Goyan et al 1979 Appl. Env. Microbiol 37, 572-575

26. Graham DY, Jiang X, Tanaka T, Opekun AR, Madore HP, Estes MK. Norwalk virus infection of volunteers: New insights based on improved assays. J Infect Dis 1994;170:34-43.

27. Gray J.J., Green J., Cunliffe C., Gallimore C., Lee J.V., Neal K., Brown D.W. Mixed genogroup SRSV infections among a party of canoeists exposed to contaminated recreational water. J Med Virol. 1997;52:425-9.

28. Green J, Norcott JP, Lewis D, Arnold C, Brown DW. Norwalk-like viruses: demonstration of genomic diversity by polymerase chain reaction. J Clin Microbiol 1993;31:3007-12.

29. Hale AD, Crawford SE, Ciarlet M, Green J, Gallimore C, Brown DW, Jiang X, Estes MK. Expression and self-assembly of Grimsby virus: antigenic distinction from Norwalk and Mexico viruses. Clin Diagn Lab Immunol. 1999;6(1):142-5.

30. Halliday M.L., Kang L.Y., Zhou T.K., Hu M.D., Pan Q.C., Fu T.Y., Huang Y.S., Hu S.L. An epidemic of hepatitis A attributable to the ingestion of raw clams in Shanghai, China. J Infect Dis. 1991;164(5):852-9.

31. Hedberg, C.W. en Osterholm, M.T. Outbreaks of food-borne and waterborne viral gastroenteritis. Clin. Microbiol. Rev. 1993;6:199-210.

32. Hedberg, C., MacDonald, K., and Osterholm, M. Changing epidemiology of food-borne disease: a Minnesota perspective. Clin Infect Dis. 1994;18:671-682.

33. Hoogenboom-Verdegaal, A.M.M., During, M., Engels, G.B., Hoekstra, J.A., Leentvaar-Kuypers, A., Peerbooms, P.G.H., Kooij, W.C.M., van Vlerken, R., en Sobczak, H. Epidemiologisch en microbiologisch onderzoek met betrekking tot gastro-enteritis bij de mens in de regio's Amsterdam en Helmond in 1987 en 1988. RIVM rapport no. 148612 002, 1990.

34. Hutin Y.J., Bell B.P., Marshall K.L., Schaben C.P., Dart M., Quinlisk M.P., Shapiro C.N. Identifying target groups for a potential vaccination program during a hepatitis A communitywide outbreak. Am J Public Health. 1999;89(6):918-21.

35. Hutin YJ, Pool V, Cramer EH, Nainan OV, Weth J, Williams IT, Goldstein ST, Gensheimer KF, Bell BP, Shapiro CN, Alter MJ, Margolis HS. A multistate, foodborne outbreak of hepatitis A. National Hepatitis A Investigation Team. N Engl J Med. 1999;340:595-602.

36. Iversen A.M., Gill M., Bartlett C.L., Cubitt W.D., McSwiggan D.A. Two outbreaks of foodborne gastroenteritis caused by a small round structured virus: evidence of prolonged infectivity in a food handler. Lancet. 1987;2(8558):556-8.

37. Jehl-Pietri CH, Hugues B, Andri M, Diez JM, Bosch A. Comparison of immunological and molecular hybridization detection methods for demonstration of hepatitis A virus in sewage. Letters in Appl Microbiol 1993;17:162-166.

38. Jiang, X., Graham DY, Wang KN, Estes MK. Norwalk virus genome cloning and characterization. Science. 1990;250:1580-3.

39. Jiang X, Wang M, Wang K, Estes MK. Sequence and genomic organization of Norwalk virus. Virology. 1993;195:51-61

40. Kapikian, A.Z., and Chanock, R.M. Norwalk group of viruses. In: Fields Virology 2nd Ed., B.N. Fields and D.M. Knipe (Eds.), Raven Press Inc. New York, NY, USA, 1990:671-693.

41. Kaplan, J.E., Feldman, R., Campbell, D.S., Lookabaugh, C., en Gary, G.W. The frequency of a Norwalk-like pattern of illness in outbreaks of acute gastro-enteritis. Am. J. Public Health 1982;72:1329-1332.

42. Keeffe E.B. Is hepatitis A more severe in patients with chronic hepatitis B and other chronic liver diseases? Am J Gastroenterol. 1995;90(2):201-5.

43. Kohn, M., Farley, T., Ando, T., Curtis, M., Wilson, S., Jin, Q., Monroe, S., Baron, R., McFarland, L., and Glass, R. An outbreak of Norwalk virus gastroenteritis associated with eating raw oysters. JAMA 1995;273:466-471.

44. Koopmans, M. Deaths asociated with outbreaks of gastrenteritis: an underestimated problem? Infect. Dis. Bull. (NL) 1997;8:251-252.

45. Koopmans, M., Vinjé, J., de Wit, M., Leenen, I., van der Poel, W., and van Duynhoven, Y. Molecular epidemiology of human enteric caliciviruses in The Netherlands. J. Infect. Dis. In press.

46. Kukkula M., Arstila P., Klossner M.L., Maunula L., Bonsdorff C.H., Jaatinen P. Waterborne outbreak of viral gastroenteritis. Scand. J. Infect. Dis. 1997;29:415-8.

47. Kukkula, M., Maunula, L. Silvennoinen, E. and C.- H. v.Bonsdorff: Outbreak of viral gastroenteritis due to drinking water contaminated by Norwalk-like viruses. J. Infect. Dis (in press) 1999.

48. Lavanchy, D., Morel, B., and Frei, P.C. Seroprevalence of hepatitis E virus in Switzerland. Lancet 1994;344:747-748.

49. Lees, D.N., Henshilwood, K., and Dore, W. Development of a method for detection of enteroviruses in shellfish by PCR with poliovirus as a model. Appl Environm Microbiol 1994;60:2999-3005.

50. LeGuyader, F. , Apaire-Marchais, V., Brillet, J., and Billaudel, S. Use of genomic probes to detect hepatitis A virus and enterovirus RNAs in wild shellfish and relationships of viral contamination to bacterial contamination. Appl Envronm Microbiol 1993;59:3963-3968.

51. LeGuyader, F., Dubois, E., Menard, D., and Pommepuy, M. Detection of hepatitis A virus, rotavirus, and enterovirus in naturally contaminated shellfish and sediment by RT-PCR. Appl Environm Microbiol 1994;60:3665-3671.

52. LeGuyader FA le, Estes MK, Hardy ME, Neill FH, Green J, Brown DW, Atmar RL. Evaluation of a degenerate primer for the PCR detection of human caliciviruses. Arch Virol 1996;141:2225-35.

53. LeGuyader F, Miossec L, Haugarreau L, Dubois E, Kopecka H, Pommepuy M. RT-PCR evaluation of viral contamination in fibe shellfish beds over a 21-month period. Water Science and Technology, 1998;2:45-50.

54. Lemon SM, Jansen RW, Brown EA. Genetic, antigenic and biological differences between strains of hepatitis A virus. Vaccine 1992;10 Suppl 1:S40-4.

55. Leoni E., Bevini C., Degli Esposti S., Graziano A. An outbreak of intrafamiliar hepatitis A associated with clam consumption: epidemic transmission to a school community. Eur J Epidemiol.1998;14(2):187-92.

56. Lew, J.F., Glass, R.I., Gangarosa, R.E., Cohen, I.P., Bern, C., and Moe, C.L. Diarrheal deaths in the United States 1979-1987. JAMA 1991;265:3280-3284.

57. Lewis DC. Three serotypes of Norwalk-like virus demonstrated by solid-phase immune electron microscopy. J Med Virol. 1990;30:77-81.

58. Lewis D, Ando T, Humphrey CD, Monroe SS, Glass RI. Use of solid-phase immune electron microscopy for classification of Norwalk-like viruses into six antigenic groups from 10 outbreaks of gastroenteritis in the United States. J Clin Microbiol. 1995;33:501-4.

59. Liu BL, Clarke IN, Caul EO, Lambden PR Human enteric caliciviruses have a unique genome structure and are distinct from the Norwalk-like viruses. Arch Virol. 1995;140(8):1345-56.

60. Liu BL, Lambden PR, Gunther H, Otto P, Elschner M, Clarke IN. Molecular characterization of a bovine enteric calicivirus: relationship to the Norwalk-like viruses. J Virol. 1999;73:819-25.

61. Lo, S.V., Connolly, A.M., Palmer, S.R., Wright, D., Thomas, P.D., en Joynson, D. The role of the pre-symptomatic food handler in a common source outbreak of food-borne NLV gastroenteritis in a group of hospitals. Epidemiol. Infect. 1994;113:513-521.

62. Low, A., McNamara, M., Schweiger, M.S. Foodborne viral gastroenteritis. Commun. Dis. Rep. CDR Rev. 1993; 3:R44-6.

63. Maguire, H.C., Handford, S., Perry, K.R., Nicholas, S., Waight, P., Parry, J.V., O'Mahoney, M., and Begg, N.T. A collaborative case control study of sproadic hepatitis A in England. CDR Review 1995;5:R33-R40.

64. Malfait P., Lopalco P.L., Salmaso S., Quarto M., Barbuti S. and Field Working Group: an Outbreak of hepatitis A in Puglia, Italy 1996. Euro Surveillance 1996; 5 : 33-35.

65. Maunula L., Piiparinen, H. and C.-H. v. Bonsdorff: Confirmation of Norwalk-like virus amplicons after RT-PCR by microplate hybridization and direct sequencing. J. Virol. Methods (in press) 1999.

66. Massoudi MS, Bell BP, Paredes V, Insko J, Evans K, Shapiro CN. An outbreak of hepatitis A associated with an infected foodhandler. Public Health Rep. 1999;114(2):157-64.

67. Mast, E.E., and Alter, M.J. Epidemiology of viral hepatitis. Seminars in Virology 1993;4:273-283.

68. Mele A., Stroffolini T., Palumbo F., Gallo G., Ragni P., Balocchini E., Tosti M.E., Corona R. , Marzolini A., Moiraghi A. and the SEIEVA Collaborating Group. Incidence and risk factor for hepatitis A in Italy: public health indications from a 10-year surveillance. Journal of Hepatology 1997; 26: 743-747.

69. Melker, H. de, van der Peet, T., Berbers, G., van de Akker, R., van Knapen, F., Schellekens, J., and Conyn-van Spaendonck, M. Pilot-study for the PIENTER project: seroprevalence of mumps, measles, rubella, Bordetella pertussis, toxoplasma gondii, Trichinella spiralis, and hepatitis A. RIVM report 213675004 (in Dutch).

70. Meng XJ, Purcell RH, Halbur PG, Lehman JR, Webb DM, Tsareva TS, Haynes JS, Thacker BJ, Emerson SU. A novel virus in swine is closely related to the human hepatitis E virus. Proc Natl Acad Sci U S A. 1997;94:9860-5.

71. Meng XJ, Halbur PG, Shapiro MS, Govindarajan S, Bruna JD, Mushahwar IK, Purcell RH, Emerson SU Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. J Virol. 1998;72:9714-21.

72. Moe, C., Sobsey, M., Stewart, P., and Crawford-Brown, D. Estimating the risk of human calicivirus infection from drinking water. Proc. 1st Int. Workshop on Human Caliciviruses, Atlanta 1999:P4-6.

73. Noel JS, Ando T, Leite JP, Green KY, Dingle KE, Estes MK, Seto Y, Monroe SS, Glass RI. Correlation of patient immune responses with genetically characterized small round-structured viruses involved in outbreaks of nonbacterial acute gastroenteritis in the United States, 1990 to 1995. J Med Virol 1997;53:372-83.

74. Noel J.S., Fankhauser R.L., Ando T., Monroe S.S., Glass R.I. Identification of a distinct common strain of "Norwalk-like viruses" having a global distribution. J Infect Dis. 1999;179(6):1334-44.

75. Pang XL, Joensuu J, Vesikari T. Human calicivirus-associated sporadic gastroenteritis in Finnish children less than two years of age followed prospectively during a rotavirus vaccine trial. Pediatr. Infect. Dis. J. 1999;5:420-6.

76. Parashar UD, Kilgore PE, Holman RC, Clarke MJ, Bresee JS, Glass RI Diarrheal mortality in US infants. Influence of birth weight on risk factors for death. Arch Pediatr Adolesc Med. 1998;152:47-51.

77. Parashar U.D., Dow L., Fankhauser R.L., Humphrey C.D., Miller J., Ando T., Williams K.S., Eddy C.R., Noel J.S., Ingram T., Bresee J.S., Monroe S.S., Glass R.I. An outbreak of viral gastroenteritis associated with consumption of sandwiches: implications for the control of transmission by food handlers. Epidemiol Infect. 1998;121:615-21.

78. Patterson W., Haswell P., Fryers P.T., Green J. Outbreak of small round structured virus gastroenteritis arose after kitchen assistant vomited. Commun Dis Rep CDR Rev 1997;7:R101-3

79. Pönkä, A, Maunula, L, v.Bonsdorff, C.-H. and O Lyytikäinen:Outbreak of calicivirus gastroenteritis associated with eating frozen raspberries. Eurosurveillance, 4, 66 - 69, 1999.

80. Purcell, R.H., and Ticehurst, J.R. Enterically transmitted non-A, non-B hepatitis: epidemiology and clinical characteristics. Viral hepatitis and Liver Disease 1988:131-137.

81. Roberts, T., and Unnevehr, L. New approaches to regulating food safety. Food Review 1994;17:2-8.

82. Robertson BH, Friedberg D, Normann A, Graff J, Flehmig B, Shouval D. Sequence variability of hepatitis A virus and factor VIII associated hepatitis A infections in hemophilia patients in Europe. An update. Vox Sang. 1994;67 Suppl 1:39-45

83. Robertson BH, Jansen RW, Khanna B, Totsuka A, Nainan OV, Siegl G, Widell A, Margolis HS, Isomura S, Ito K, et al. Genetic relatedness of hepatitis A virus strains recovered from different geographical regions. J Gen Virol. 1992;73:1365-77.

84. Rosenblum L.S., Villarino M.E., Nainan O.V., Melish M.E., Hadler S.C., Pinsky P.P., Jarvis W.R., Ott C.E., Margolis H.S. Hepatitis A outbreak in a neonatal intensive care unit: risk factors for transmission and evidence of prolonged viral excretion among preterm infants. J Infect Dis. 1991;164(3):476-82.

85. Sjogren M.H., Tanno H., Fay O., Sileoni S., Cohen B.D., Burke D.S., Feighny R.J. Hepatitis A virus in stool during clinical relapse. Ann Intern Med. 1987;106(2):221-6.

86. Skala, M., Collier, C., Hinkle, C.J., Donnell, H.D. Foodborne hepatitis A - Missouri, Wisconsin, and Alaska, 1990-1992. MMWR 1993;42:526-529.

87. Sobsey, M., Shin, G.-A., Battigelli, D., and Newland, S. Reduction of Norwalk virus by drinking water treatment processes. Proc. 1st Int. Workshop on Human Caliciviruses, Atlanta 1999:P4-3.

88. Sugieda M., Nakajima K., Nakajima S. Outbreaks of Norwalk-like virus-associated gastroenteritis traced to shellfish: coexistence of two genotypes in one specimen. Epidemiol Infect. 1996;116:339-46.

89. Sugieda M, Nagaoka H, Kakishima Y, Ohshita T, Nakamura S, Nakajima S. Detection of Norwalk-like virus genes in the caecum contents of pigs. Arch Virol. 1998;143:1215-21.

90. Taylor. Molecular epidemiology of South African strains of hepatitis A virus: 1982-1996. J Med Virol 1997;51:273-299.

91. Trautwein, C., Kiral, G., Tillmann, H.L., Witteler, H., Michel, G., and Manns, M.P. Risk factors and prevalence of hepatitis E in German immigrants from the former Soviet Union. J. Med. Virol. 1995;45:429-434.

92. Vandenvelde, C., Heyvaert, F., Maes, F., Kadi, L., and Opstelten, R. Hepatitis E virus infection in Belgian soldiers. Lancet 1994;344:747.

93. Vento S., Garofano T., Renzini C., Cainelli F., Casali F., Ghironzi G., Ferraro T., Concia E. Fulminant hepatitis associated with hepatitis A virus superinfection in patients with chronic hepatitis C. N Engl J Med. 1998;338(5):286-90.

94. Vinjé, J., and Koopmans, M. Molecular detection and epidemiology of NLV in outbreaks of gastroenteritis in The Netherlands. JID 1996;174:610-615.

95. Vinjé, J., Altena, S., and Koopmans, M. The incidence and genetic variability of small-round-structured viruses (SRSV) in outbreaks of gastroenteritis in The Netherlands. JID 1997;176:1374-1378.

96. Wait et al. 1983 J. Food Prot. 46, 493-497

97. Wheeler, J.G., Sethi, D., Cowden, J.M., Wall, P.G., Rodrigues, L.C., Tompkins, D.S., Hudson, M.J., Roderick, P.J. Study of infectious intestinal disease in England: rates in the community, presenting to the general practitioner, and reported to national surveillance. BMJ 1999;318:1046-50.

98. Zhang, X.M., Herbst, W., Kousoulas, K.G., en Storz, J. Biological and genetic characterization of a hemagglutinating coronavirus isolated from a diarrhoeic child. J. Med. Virol. 1994;44:152-161.

99. Anon. Small round structured viruses (SRSV): numbers are increasing. Commun. Dis. Rep. CDR Wkly. 1995;5(23): 10.