Viruses as a cause of foodborne diseases: a review of the literature

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ABSTRACT: Viruses cause many diseases in plants, animals, and humans. They are strict intracellular parasites with cellular specificity. Viral particles can be transmitted by different routes, such as contaminated food and water. People usually get infected orally, after ingestion of products contaminated during processing or subsequent handling or preparation. This review article is focused on the most severe foodborne viruses specific for humans, of the following genera: *Norovirus, Enterovirus, Hepatovirus, Astrovirus*, and some others. Methods for detecting viruses in food and strategies for preventing virus transmission via food are also discussed.

Keywords: risk assessment; food safety; enteric viruses; route of transmission; RT-PCR; ELISA; foodborne viral outbreaks; zoonoses

Contents

1. Introduction

The spectrum of foodborne pathogens includes a variety of entric bacteria, aerobes and anaerobes, viral pathogens, and parasites, as well as marine dinoflagellates, bacteria that produce biotoxins in fish and shellfish, and the self-inducing prions of transmissible encephalopathies (Tauxe, 2002).

Viruses are the most common pathogens transmitted via food, for example they cause 66.6% of food-related illnesses in the United States, compared to 9.7% and 14.2% for salmonella and campylobacter, respectively (Mead et al., 1999). In the Europe the Norwalk-like caliciviruses and hepatitis A virus are currently recognised as the most important human foodborne pathogens with regard to the number

of outbreaks and people affected (Cliver, 1997a). For example, in the Netherlands, approximately 80% of outbreaks of gastroeteritis reported to municipal health services are caused by Norwalk-like viruses. In 2003 in the Czech Republic no case of Norwalk-like viruses infection was detected, but epidemiologic significance of rotavirus infections is growing (Drapal et al., 2003). Viruses (especially Norwalk-like viruses and hepatitis A) are highly infectious and may lead to widespread outbreaks. Most documented foodborne viral outbreaks can be traced to food manually handled by an infected foodhandler, rather than to industrially processed foods (Bidawid et al., 2000).

Viruses are small acellular microorganisms with diameters of 15 to 400 nm, each containing only one type of nucleic acid. They cause many diseases of plants, animals and humans. They are strict intracellular parasites with cellular specificity (cell tropism). Their replication is strongly dependent on the host organism; they cannot multiply outside the host. Viruses can be transmitted in different ways, for example (Bednar et al., 1999; Koopmans and Duizer, 2004):

– Aerosol
– Subjects soiled with human or animal faeces or vomit
– Contact with blood of infected persons
– Contact with diseased animals
– Sexual intercourse
– Vectors such as gnats or ticks that can transmit arboviruses

Numerous viruses can be found in human gut, but only a few are commonly recognised as important foodborne pathogens. Original foodborne pathogens are always transmitted through food, while the others are capable of being transmitted via several different routes in addition to food. The *Norovirus* and hepatitis A virus are currently recognised as the most important human foodborne pathogens with regard to the number of outbreaks and people affected in the Western world. Some large foodborne outbreaks have occurred with group B and C rotaviruses, and waterborne outbreaks have occurred with hepatitis E virus (Cliver, 1997a; Tauxe, 2002; Koopmans and Duizer, 2004). In the review causal agents of viral infections are also mentioned, which are not included in foodborne viral infections, but which are transmitted through food acting as *vehiculum*.

Viruses causing foodborne diseases attack cells of the digestive tract and propagate inside them;

subsequently they attack other cells of the digestive tract or enter other organs such as the liver or central nervous system and cause disease. The primary symptoms of viral foodborne diseases and differences from bacterial infections are as follows:

(i) Only a few viral particles are necessary for the disease to develop (Bajolet and Chippaux-Hyppolite, 1998; Koopmans and Duizer, 2004)
(ii) High numbers of viral particles are further transmitted via faeces of infected people (up to 10^{11} particles per gram of faeces in members of genus *Rotavirus*)
(iii) Specific living cells are necessary for virus replication; accordingly they cannot multiply in foods or water
(iv) Foodborne viruses are relatively stable and acid-resistant outside host cells (Koopmans and Duizer, 2004)

2. Aetiology of foodborne human viral infections

According to data from 10 surveillance systems of the Foodborne Viruses in Europe network the *Norovirus* is found to be responsible for > 85% (n = 3.714) of all nonbacterial outbreaks of gastroenteritis reported from 1995 to 2000 in Europe (Lopman et al., 2003). Hepatitis A is an increasing problem in the Western countries of Europe because of the decrease in immunity of population in countries with high standard of hygiene. Most foodborne viruses are more resistant to heat, desinfection, and pH changes than are most vegetative bacteria (Koopmans and Duizer, 2004).

People commonly get infected by eating products that have been contaminated during processing. Contamination may occur through:

(i) Contact with human or animal faeces, or water contaminated with faeces (washing, irrigation, etc.)
(ii) Contact with hands, objects soiled with faeces
(iii) Contact with vomitus or water contaminated with vomit
(iv) Contact with the environment where infected persons were previously present
(v) Virus-containing aerosol produced by infected persons (Cliver, 1997a; Koopmans and Duizer, 2004)

The extent and incidence of contamination may vary between products. Humans can become in-

fected by eating products such as meat or milk originating from a previously infected animal (Acha and Szyfres, 2003).

Viruses are strict intracellular parasites and cannot replicate outside a specific living cell. The host cell treats viral genetic information as if it were its own. Replication of viruses occurs by transcription and translation of the viral genome using host cell mechanisms. It is not possible to culture them in an environment free of living cells, and therefore the number of viral particles does not increase in food and water during production, processing, transport, and storing. Sensory characteristics of products containing these pathogens and those of non-contaminated food are identical (Cook, 2001; Koopmans and Duizer, 2004). Transmission of the virus does not only depend on its interaction with the host, but also on the influence of the external environment. Outside the host organism, viruses are inert particles without their own metabolism. The longer they survive in the infectious state environment, the higher is the probability of transmission and spread of infection (Rzezutka and Cook, 2004).

2.1. Genera *Norovirus* and *Sapovirus*

New names for two of the four genera of the Caliciviridae were approved in 2002. They are *Norovirus*, for what was previously called Norwalk-like viruses or small, round-structured viruses, and *Sapovirus*, for what was previously called Sapporo-like viruses (Matson and Szucs, 2003). The viral genome contains single-stranded plus sense RNA. These viruses do not grow in cell or organ culture and there is no animal model for *Norovirus* infection and gastrointestinal disease. Without an infection model current knowledge of Norwalk virus infection and disease is derived from outbreaks and volunteer challenge studies. Research into both the genera has increased through advances in molecular biological detection and confirmation procedures (Hutson et al., 2004).

The first recognized *Norovirus*, Norwalk virus, gained its name from an outbreak of "winter vomiting disease" in 1968 at an elementary school in Norwalk, Ohio in the USA (Adler and Zickl, 1969). Noroviruses are frequently the cause of sporadic cases and also outbreaks of acute gastroenteritis in children and adults (Kaplan et al., 1982; Kapikian, 1996; Vinje and Koopmans, 1996; Caul, 1996a,b;

Hedlund et al., 2000) particularly in semi-closed environments such as schools, cruise ships, hospitals and residential homes (Lopman et al., 2002). These pathogens had been viewed as exclusively human; however, viruses similar in morphological and molecular aspects have been detected in cattle (Dastjerdi et al., 1999; Liu et al., 1999) and pigs (Sugieda et al., 1998).

Factors that contribute to the significant impact of noroviruses include a large human reservoir, low infection dose (only 10 to 100 virions can cause the disease), their environmental robustness, the short-lived immunity to noroviruses (18 months max.), and the ability to be transmitted by various routes. Water may be a vector (in swimming pools, occasionally non-sufficiently treated potable water) and any food handled by an infected person (Dubois et al., 2002; Votava et al., 2003; Koopmans and Duizer, 2004). Viruses are present in faeces and vomitus of diseased people (Bednar et al., 1999). The foods most closely associated with noroviral infection are shellfish which obtain their nourishment by filtration of surrounding water and thus they ingest small particles such as seaweeds and other microorganisms including viruses, the later being concentrated within the gills of the shellfish (Lees, 2000).

An outbreak of Norwalk virus gastroenteritis following consumption of oysters was described in Queensland in Australia (Stafford et al., 1997). Ninety-two of the 97 cases identified were confirmed as having consumed raw oysters within three days prior to developing the illness. Kirkland et al. (1996) reported that the risk of illness increased with the number of oysters eaten and the even steaming oysters was not adequate to inactivate these viruses and to prevent illness.

The clinical manifestation of *Norovirus* infection, however, is relatively mild. The symptoms are vomiting and diarrhoea, and (rarely) convulsion and others. Asymptomatic infections are common and may contribute to the spread of the infection (Ushijima, 2002). Introduction of *Norovirus* in a community or population (a seeding event) may be followed by additional spread of the disease because of its highly infectious nature, resulting in a large number of secondary infections, up to 50% of contacts (Koopmans and Duizer, 2004). This virus is currently recognized as the cause of almost all (> 96%) outbreaks of non-bacterial gastroenteritis in adults (Mead et al., 1999), particularly in Europe and Australia where there is active surveillance. *Norovirus* is also the cause of an

estimated 23 million cases of gastroenteritis per year in the USA (Franhauser et al., 1998; Mead et al., 1999; Lopman et al., 2002).

An outbreak of acute gastroenteritis associated with noroviruses among students at the Texas University (USA) was described by Daniels et al. (2000). Stool specimens from 9 (50%) of 18 ill students and samples of deli ham from the university's main cafeteria deli bar showed evidence of Norwalk-like viruses. Reverse-transcriptase polymerase chain reaction (RT-PCR) successfully confirmed that the food was contaminated by a food handler during preparation of sandwiches. In the Czech Republic, the year 2001 showed the highest number of noroviral infections, with 104 cases recorded. In 2003 however, there were only small outbreaks in nursing home for pensioners in Prague (Drapal et al., 2003).

Sero-epidemiologic studies in adult people showed the worldwide spread of members of genus *Sapovirus* (Nakata et al., 1996 as quoted by Lees, 2000). Saproviruses mainly affect babies and children under the age of five years in whom they may induce inapparent infection. Infections are not associated with eating seafood (Lees, 2000). Mixed infections with sapoviruses, astroviruses, and noroviruses have been recorded in faeces of children with acute gastroenteritis in Argentina (Martinez et al., 2002), England (Robinson et al., 2002), Finland (Pang et al., 2001), Hungary (Reuter et al., 2002), Japan (Sakai et al., 2001), the Netherlands (Koopmans et al., 2001), Pakistan (Phan et al., 2004), Russia (Mukhina et al., 2002), and Spain (Buesa et al., 2002).

2.2. Genus *Enterovirus*

The genus *Enterovirus* is a member of the broad Picornaviridae family of RNA viruses. The genus *Enterovirus* is divided into five major groups: polioviruses, group A coxsackieviruses, group B coxsackieviruses, echoviruses and newer identified enteroviruses. The human enteroviruses are ubiquitous, enterically transmitted viruses that cause a wide spectrum of illnesses among infants and children (Acha and Szyfres, 2003; Cliver, 2000). Designation of enteroviruses is used due to their capability to multiply in the intestine. They are quite resistant to the impact of the environment where they can survive for several weeks; they are stable in acid conditions (pH 3 to 5), and consequently also in gastric juices (Votava et al., 2003). Kurdziel et al.

(2001) performed a study, using poliovirus, to ascertain the potential for enteric pathogenic viruses to survive on various foodstuffs. The results showed that enteric viruses may persist on fresh fruit and vegetables for several days under conditions commonly used for storage in households. Therefore, there will be a risk of infection from consumption of those foods if they are contaminated with viruses.

Though enteroviruses are particularly transmitted via the faecal-oral route, the spread of certain species via aerosol is also a cause for concern (Bednar et al., 1999). Viral particles are shed with faeces and symptoms of the diseases caused by them are different from typical gastroenteritis. Viruses enter the host with contaminated water or food and multiply in the digestive tract. Symptoms of infection are often slight, moderate but almost the enterovirus infections are asymptomatic. However, viruses may spread into other organs and cause diseases that are serious or even fatal such as aseptic meningitis, and occasionally paralysis (Lees, 2000).

Cases of infection giving evidence of association with eating soft fruit, green vegetables and other foods have been recognised (Koopmans and Duizer, 2004; Cook and Rzezutka, 2005). Enteroviruses have been frequently isolated from shellfish samples, particularly from oysters. The count of contaminated samples ranged from 19 to 63% (Le Guyader et al., 1993, 1994; Beuret et al., 2003; Muniain-Mujika et al., 2003). When these viruses were detected in sediments, they were also detected in shellfish (Le Guyader et al., 1994).

2.3. Genus *Hepatovirus*

Hepatitis A virus (HAV) is the only member of the genus *Hepatovirus* of the family Picornaviridae. HAV occurs as a single antigenic type; nonetheless, four human genotypes, and three genotypes naturally affecting other primates (chimpanzee and non-human primates) can be discriminated. HAV differs from enteroviruses by certain biological characteristics such as marked tropism for liver cells, exceptional thermostability (it survives heating for 30 min to 56°C), acid-resistance (it tolerates pH 1) or slow replication without cytopathic effect on the host cell (WHO, 1993; Cromeans et al., 1994; Bednar et al., 1999).

The virus is most commonly transmitted via the faecal-oral route, either by direct contact with an HAV-infected person or by ingestion of HAV-contaminated

food or water (Cromeans et al., 1994; Cliver, 1997b; Fiore, 2004; Keeffe, 2004). Foodborne or waterborne HAV outbreaks are uncommon in the USA. However, food handlers with HAV are frequently identified, and the need for immunoprophylaxis and implementation of control measures are a considerable burden on public health resources. In addition, HAV-contaminated food may be the source of HAV for an unknown proportion of persons whose source of infection is not identified (Fiore, 2004). The last large outbreak of hepatitis in the Czech Republic occurred in the year 1979 when 28 880 persons became ill. The outbreak was associated with frozen products made of imported strawberries watered with sewage (Votava et al., 2003). Washing of contaminated fresh vegetables does not guarantee elimination of viral particles (Croci et al., 2002).

The Pennsylvania Department of Health and Central Disease Control (USA) have investigated an outbreak of HAV among patrons of a Restaurant "A" in Monaca (Pennsylvania, USA) in 2003. Since November 20, 2003, approximately 555 persons with HAV have been identified, including at least 13 Restaurant "A" food service workers and 75 residents of six other states who dined at Restaurant "A". Three persons died. Preliminary sequence analysis of a 340 nucleotide region of viral RNA obtained from three patrons who had HAV indicated that all three virus sequences were identical. Preliminary analysis of a case-control study implicated green onions as the source of the outbreak (Dentinger et al., 2001). Based on molecular fingerprinting, an outbreak of infection with HAV associated with a food handler and involving 26 subjects was analysed in southern Italy (Chironna et al., 2004).

Likewise shellfish are readily contaminated with viruses HAV present in water containing sewage because of the concentration effect of filter feeding (Formiga-Cruz et al., 2002; Coelho et al., 2003; Di Pinto et al., 2004). RT-PCR method was used to assess the viral contamination of four shellfish beds in Santa Catarina State (Brazil), over a 1-year period. Six (22%) of 27 samples collected in autumn and winter from one shellfish bed tested positive for HAV (Coelho et al., 2003).

2.4. Hepatitis E virus

Hepatitis E virus (HEV) is a small non-enveloped virus, which was previously classified as a member of the Caliciviridae family, but recent data based on genome organization and nucleotide sequence analysis has revealed differences, so it is now provisionally classified in a separate genus "HEV-like viruses" (Jameel, 1999; Berke and Matson, 2000). The existence of this non-A, non-B hepatitis virus was confirmed, and its non-segmented ssRNA genome was cloned and sequenced in 1991 (Molinie and Desrame, 1996). HEV is a major cause of outbreaks and sporadic cases of viral hepatitis in tropical and subtropical countries but is infrequent in industrialized countries. The virus is transmitted by the faecal-oral route with faecal contaminated drinking water being the usual vehicle. Direct contamination is rare. Young adults, 15 to 30 years of age, are the main targets of infection, and the overall death rate is 0.5 to 3.0%. The disease is usually mild, except in pregnant women, who suffer a high fatality rate from fulminant hepatic failure (Molinie and Desrame, 1996; Smith, 2001).

Prevalence of antibodies among blood donors in Europe is about 1%, HEV is also found in both wild and domestic animals; accordingly, it is a zoonotic virus. Antibodies have also been detected in pigs, rodents, and other animals (Favorov et al., 2000; Smith, 2001). The close genetic relationship of the swine and human virus suggests that swine may be a reservoir of HEV and swine manure could be a source of HEV contamination of irrigation water or coastal waters with concomitant contamination of produce or shellfish (Smith, 2001). Cacopardo et al. (1997) identified travel in the tropics and shellfish ingestion as risk factors for HEV transmission.

Consumption of uncooked deer meat was a major epidemiological risk factor for HEV infection in Kasai, a city in western Japan (Tei et al., 2004). In total, 45 volunteer subjects with experience of eating raw deer meat were enrolled. An equivalent number of people from the same area who had never eaten raw deer meat served as controls. Eight (17.7%) of the subjects but only one (2.2%) of the controls had measurable serum anti-HEV IgG levels ($P = 0.014$). The results suggest that eating uncooked deer meat is an epidemiological risk factor for HEV infection in the studied area. An outbreak of acute icteric hepatitis caused by hepatitis E virus associated with food intake was described in China (Tan et al., 2003).

The studies of Yazaki et al. (2003) and Tamada et al. (2004) suggest that consumption of undercooked pig liver, and undercooked wild boar meat may have been the cause of some cases of hepatitis E in Japan.

Wild boar liver is also often eaten raw in Japan, and this has also been linked to some hepatitis E cases (Matsuda et al., 2003). In Bali, raw pig meat or fresh pig blood can be consumed, and seropositivity to HEV is relatively high in the human population (Wibawa et al., 2004). In a case of hepatitis E in the UK which was caused by an HEV strain very similar to pig strains, the patient had admitted to eating raw pork products, although this was not conclusively the cause of the infection (Banks et al., 2004).

2.5. Genus *Astrovirus*

Astroviruses are small, 28 nm diameter non-enveloped, single-stranded RNA viruses comprising the only members of the family Astroviridae. Human astrovirus is a significant cause of acute diarrhea among children, resulting in outbreaks of diarrhea and occasionally in hospitalization. Astrovirus disease is generally milder than that caused by rotaviruses. However, frequent coinfection of astrovirus with rotavirus and caliciviruses in childhood diarrhea complicates the epidemiology. Infections are more common in winter. Non-enteric symptoms can often be observed in grown up children on numerous occasions (subfebrility, headache etc.). A number of cases go unnoticed (Kurtz and Lee, 1987; Walter and Mitchell, 2000). The likely route of transmission is faecal-oral via food or water (Yamashita et al., 1991; Walter and Mitchell, 2000). Nevertheless, a direct epidemiological link between eating raw food and astrovirus infection has not been fully demonstrated. Easier availability of molecular diagnostic methods may provide new approaches to clarification of these problems (Lees, 2000).

Wading pool water contaminated with both noroviruses and astroviruses was determined as the source of a gastroenteritis outbreak in Finland (Maunula et al., 2004). The epidemiological survey revealed that at least 242 persons were affected (Maunula et al., 2004). The pathogenic enteric viruses including astroviruses were detected by RT-PCR and hybridization in shellfish (Le Guyader et al., 2000). Mussel samples (50%) were more highly contaminated with astroviruses than oyster samples (17%). Viral contamination was mainly observed during winter months, although there were some seasonal differences among the viruses.

2.6. Genus *Rotavirus*

Rotaviruses belong to the family Reoviridae, they are segmented double-stranded RNA viruses, which explains their genetic variability and the presence of mixed infections. According to the group and subgroup specific antigen, this genus is antigenically divided into serological groups, from A (with two to three subgroups and 11 serotypes) to E; *Rotavirus* F and *Rotavirus* G groups are provisional for the present (Acha and Szyfres, 2003). Groups A, B, and C of human rotaviruses have been recognized. Segmentation of the genome allows genomic reassortment, which results in development of new antigenic types (Votava et al., 2003). The viruses are not enveloped, and thus have a degree of robustness in the environment outside of a host (Bajolet and Chippaux-Hyppolite, 1998). Rotaviruses can survive for weeks in potable and recreational waters and for at least four hours on human hands. The viruses are relatively resistant to commonly used hard-surface disinfectants and hygienic hand-wash agents (Ansari et al., 1991).

Their massive excretion (10^8 to 10^{11} viral particles per gram of faeces) begins with the first day of diarrhoea (Bajolet and Chippaux-Hyppolite, 1998; Koopmans and Duizer, 2004). They are found in waste water and can also be concentrated by shellfish, however, rotaviruses have not been linked with infectious disease following seafood consumption (Cook et al., 2004; Lees, 2000). Rotavirus is transmitted by faecal-oral contact and possibly by contaminated surfaces and hands and respiratory spread. Oral-faecal transmission is facilitated by deficient sanitary conditions (Bajolet and Chippaux-Hyppolite, 1998; Dennehy, 2000; Cook et al., 2004). Numerous animal species are infected with rotaviruses distinct from the human ones. Between human and animal rotaviruses there can be genetic reassortment though the VP6 antigen remains common in the group. Reassortant viruses with animal rotavirus sequence characteristics have been isolated from humans (Bajolet and Chippaux-Hyppolite, 1998; Okitsu-Negishi et al., 2004).

Human rotaviruses, particularly of group A, are considered the main cause of viral gastroenteritis in infants and young children (from six months to three years of age) throughout the world, but the mortality rate is high only in developing countries (Bajolet and Chippaux-Hyppolite, 1998). Antibodies against these viruses are present almost in all children under five years of age, though they

cause mortality in up to 20% of children in developing countries (Matsumoto et al., 1989).

Rotaviruses of group B cause gastroenteritis in adult persons (Bridger, 1994 as quoted by Lees, 2000). In 1999 an outbreak of group A rotavirus acute gastroenteritis was recorded in university students in the District of Columbia (DC), USA. Some ill students reported eating tuna or chicken salad sandwiches from dining hall "A" on campus (Anonymous, 2000b). Mikami et al. (2004) described an outbreak of acute gastroenteritis involving 45 school children out of a total of 107 (aged 11 to 12 years) attending a 3-day school trip in Japan in 2001. Epidemiological and virological investigations concluded that this outbreak was caused by a single strain of serotype G2 group A rotavirus spreading to schoolmates from the primary case pupil who had already been ill at the start of the trip.

Rotaviruses of group C were the cause of an outbreak of acute gastroenteritis which occurred in April 1988 among schoolchildren and their teachers simultaneously at seven elementary schools in Fukui City (Japan). Of 3 102 675 became ill 676 383 (21.8%). The probable source of infection was food from the school canteen (Matsumoto et al., 1989). A similar case with no identified food cause was found by epidemiological analysis of the outbreak in 1991 in Tokyo (Sekine et al., 1993) and in 2000 in youth educational centre located in the southern area of Okayama Prefecture (Kuzuya et al., 2003).

Rotaviral infection may develop directly after consumption of meat from an infected animal, or indirectly by consumption of contaminated food usually eaten raw (fruit and vegetables) (Richards, 2001). Food contaminated after being cooked may also be the source of viral infection (Svensson, 2000; Cook, 2001; Cook et al., 2004). Incidence of rotaviral infections in the Czech Republic has been sharply increasing. Notified incidence in the year 2003 (1 541 cases) was almost double that in the year 2000 (Drapal et al., 2003).

2.7. Family Adenoviridae

The family Adenoviridae is comprised of four genera. Human and some animal adenoviruses are members of genus *Mastadenovirus.* Adenoviruses are middle-sized (80 nm) non-enveloped DNA viruses; their virions show the shape of an ideal regular icosahedron under electron microscopy (Votava et al., 2003).

Adenoviruses can spread not only via droplet infection, but also via the faecal-oral route. They cause 10% of gastroenteritis in children and are the second most common cause of hospitalization due to diarrhoea of children in Japan (Araki et al., 1994). In Finland enteric adenoviruses were found in 6% of the cases of children between 2 months and 2 years of age (Pang et al., 2000). Together with adenoviruses causing respiratory diseases they can be isolated from faeces of affected children. They may be detected in sewage, sea water and shellfish (Girones et al., 1995; Pina et al., 1998; Vantarakis and Papapetropoulou, 1998). Adenoviruses were detected in 18.6% of the shellfish samples from the Norwegian coast with more positive samples in the winter (Myrmel et al., 2004). Also Muniain-Mujika et al. (2003) examined the presence of human pathogenic viruses in shellfish and human adenoviruses were found in 47% of the samples. It was found that all the samples positive for enterovirus and HAV were also positive for human adenovirus. The human adenoviruses detected by PCR correlate with the presence of other human viruses and could be useful as a molecular index of viral contamination in shellfish (Formiga-Cruz et al., 2002).

Incidence of adenoviral infections in the Czech Republic is low; 285 cases were recorded in the year 2003 (Drapal et al., 2003).

2.8. The other viruses transmissible via food

These viruses are capable of being transmitted via several different routes in addition to food and they are not originally classified like foodborne viruses. The case made for these viruses is very poor, but due to the fact that these pathogens include the causal agents of serious infections (f.e. genus *Flavivirus*), these viruses should be kept in mind.

2.8.1. Genus *Arenavirus*

Arenaviruses are middle sized (110 to 130 nm) enveloped RNA viruses, densely covered with protuberances. They are members of family Arenaviridae. Several arenaviruses cause viral hemorrhagic fever syndrome in Africa and South America, but they are not common causes of disease in the developed world (Webb et al., 1986). The South America hemorrhagic fever viruses belong to the Tacaribe complex or New World arenaviruses

(e.g. Guanarito in Venezuela, Junin in Argentina, Machupo in Bolivia). The Old World arenaviruses include the agents of Lassa fever and lymphocytic choriomeningitis (Bednar et al., 1999; Anonymous, 2000a).

With the exception of viruses of the Tacaribe complex (with bats being reservoirs) rodents are natural sources of arenaviruses. Infection can be transmitted to man by contact with infected animals or their excretions. The virus can penetrate the body through injured skin, by consumption of contaminated food or by aerosol that contacts the conjunctiva and oral or nasal mucosa. Person-to-person transmission has been only rarely documented for some New World viruses. Preventive measures for arenavirus infections include control and exclusion of rodents in and around human dwellings and disinfection of areas and surfaces potentially contaminated by rodent excreta (Bednar et al., 1999; Anonymous, 2000a; Acha and Szyfres, 2003; Votava et al., 2003).

In a population-based study of German scientists possible risk factors for rodent-to-human transmission of Lassa virus were correlated with markers of Lassa fever in two different regions of the Republic of Guinea: Prefectures of Pita and Gueckedou (Ter Meulen et al., 1996). Antibody prevalence was 2.6% (6 of 232) in Pita compared with 14.0% (105 of 751) in Gueckedou. They observed three major risk factors in Gueckedou favouring Lassa virus transmission:

(i)   Rodent infestation was much higher
(ii) Food was more often stored uncovered
(iii) Most strikingly, peridomestic rodents were hunted as a protein source by 91.5% of the population as opposed to 0% in Pita

Rodent consumption was analyzed as a risk factor for transmission of Lassa virus comparing rodent consumers and non-consumers (Ter Meulen et al., 1996).

2.8.2. Genus *Flavivirus*

The members of genus *Flavivirus* are 40 to 60 nm in diameter enveloped viruses with many minuscule protuberances. Their genome comprises single-stranded, non-segmented, positive-sense RNA. Many viruses formerly designated as B group arboviruses are included in this genus. According to antigenic relationships, several antigenic groups have been recognized amongst the flavoviruses:

– Tick-borne encephalitis virus group (TBE)
– Yellow fever virus group
– Dengue group etc.

These arboviruses occur all over the world. Their vectors are particularly gnats, with the exception of the tick-borne encephalitis virus group.

TBE is transmitted to humans usually by the bite of a tick (either *Ixodes persulcatus* or *Ixodes ricinus*); occasionally, cases occur following consumption of infected unpasteurized milk (Dumpis et al., 1999; Acha and Szyfres, 2003; Votava et al., 2003). TBE is a serious cause of acute central nervous system disease, which may result in death or long-term neurological sequelae. Effective vaccines are available in a few countries (Dumpis et al., 1999). Labuda et al. (2002) monitored the occurrence of TBE virus in ticks and vertebrate hosts including humans in Slovakia.

Numbers of diagnosed hospitalised cases of TBE varied from less than 20 to almost 100 cases annually with 54 to 89 cases in recent years. A part of these cases (33 cases during the last five years) were alimentary infections after drinking of raw goat and sheep milk. A family outbreak of TBE in Slovakia after consumption of unboiled goat milk was described by Kohl et al. (1996). A similar case with TBE which occurred in Russia developed as a result of drinking raw cow milk (Vereta et al., 1991).

2.8.3. Genus *Hantavirus*

Hantavirus is a RNA-virus of the family Bunyaviridae that is found in the urine, saliva, or droppings of infected deer mice and some other wild rodents. Hantaviruses have been identified as etiologic agents of two human diseases. They may cause a rare but serious lung disease called Hantavirus pulmonary syndrome (HPS) and pathogenic European hantaviruses (Puumala, Doll, and Saaremaa viruses) can cause a human disease designated "hemorrhagic fever with renal syndrome" of varying severity (Vapalahti et al., 2003; Dekonenko and Tkachenko, 2004).

People can contract the Hantavirus infection through inhalation of respirable droplets of saliva or urine, or through the dust of faeces from infected wild rodents, especially the deer mouse. Transmission can also occur when contaminated material gets into broken skin, or possibly, ingested in contaminated food or water (Acha and Szyfres, 2003; Votava et al., 2003; Dekonenko and

Tkachenko, 2004; Ulrich et al., 2004). Duration of viraemia in infected rodents and virus survival in tissues indicate that contamination of the ambient environment with excretions and secretions could be prolonged (Lee et al., 1981 as quoted by Acha and Szyfres, 2003).

Many outbreaks of Hantavirus infections have been described in Europe and other continents; its occurrence was associated with infestation of rodents detected in those areas (Olsson et al., 2003; Pini et al., 2003; Ruedas et al., 2004; Ulrich et al., 2004). According to study by Van Loock et al. (1999) professional activity (sighting of living rodents, exposure to rodent droppings, and trapping rodents) appears to be a more important risk factor for acquisition of *Hantavirus* in Europe.

2.8.4. Foot-and-Mouth Disease virus

The agent responsible for Foot-and-Mouth Disease (FMD) virus is a RNA virus of the genus *Aphthovirus* that belongs to the family Picornaviridae. FMD is a zoonosis, a disease transmissible to humans, but it crosses the species barrier with difficulty and with little effect. Given the high incidence of the disease in animals, its occurrence in man is rare (Prempeh et al., 2001; Tomasula and Konstance, 2004). The last human case reported in Britain occurred in 1966 (Armstrong et al., 1967). No human cases were reported during the outbreak in UK in 2001 (Cook, 2001).

The virus is shed into milk up to 33 h before there are apparent signs of the disease in dairy cows, and, in extreme cases, signs of disease may not appear for up to 14 days. During this time, raw milk can serve as a vector for spread of the disease both on the farm and during transport to the processing plant by milk tanker. Raw milk and milk products fed to animals have the potential to cause infection, but the potential for pasteurized milk products to cause infection is largely unknown. FMD virus can survive for a long time in fresh, partially cooked, cured, and smoked meat and in inadequately pasteurised dairy products (Tomasula and Konstance, 2004).

Transmission to man usually occurs as a result of the consumption of unprocessed milk or as a result of direct contact with infected animals. Person to person spread has not been reported. Symptoms of FMD in humans include malaise, fever, vomiting, red ulcerative lesions (surface-eroding damaged spots) of the oral tissues, and sometimes vesicular lesions (small blisters) of the skin (Prempeh et al., 2001; Lopez-Sanchez et al., 2003). The type of virus most frequently isolated from man is type O followed by type C and rarely A. The incubation period in man, although somewhat variable, has not been found to be less than two days and rarely more than six days (Bauer, 1997). The treatment of FMD is essentially symptomatic, and prophylactic measures comprise the avoidance of unboiled milk and of close contact with potentially infected animals (Lopez-Sanchez et al., 2003).

2.8.5. *Aichi* virus

Aichi virus, the type species of a new genus, *Kobuvirus*, of the family Picornaviridae (Yamashita et al., 1998; Sasaki et al., 2001), was first recognised in 1989 as the cause of oyster-associated non-bacterial gastroenteritis in man (Yamashita et al., 1991). Cytopathic agents were isolated in faecal samples from 3 of 12 patients. The isolates were approximately 30 nm in diameter and had a distinct ultrastructure resembling that of astroviruses. No cross-neutralizing reactions were observed between the isolates and prototypes of human enteroviruses. *Aichi* strain could be a new type of small round virus that mainly produces diarrhea in patients in the 15- to 34-year-old age group, 50 to 76% of whom possess neutralizing antibody.

Antibody surveys in Japan showed that exposure increased with age reaching about 80% in persons 35 years old (Yamashita et al., 1993). More recently *Aichi* virus was isolated from 5 (2.3%) of 222 Pakistani children with gastroenteritis, but none was found in 91 healthy children. *Aichi* virus was also isolated from 5 (0.7%) of 722 Japanese travellers returning from tours of Southeast Asian countries (Indonesia, Thailand and Malaysia) who were complaining of similar symptoms (Yamashita et al., 1995). These results indicate that *Aichi* virus or a similar agent is endemic in Southeast Asian countries.

3. Methods for detecting viruses in food

Although viral foodborne disease is a significant problem, foods are rarely tested for viral contamination, and when done, testing is limited to shellfish commodities. It is difficult to culture the most important foodborne viruses in cell cultures; they must be directly detected in food extracts, which

is accompanied with many problems concerning standardization, inhibition of enzymes used in RT-PCR, false positive results etc. (Lees, 2000; Atmar et al., 2001 as quoted by Koopmans and Duizer, 2004). Foods are most usually contaminated by food-handlers who prepare them. Accordingly, the contamination degree can be variable even in identical products.

Infection with gastroenteric viruses is routinely diagnosed by examination of stool samples by electron microscopy. Particularly with human rotaviruses, when high numbers of virus particles are present in faeces during the acute gastroenteritis, methods based on electron microscopy, but also passive particle agglutination tests, or enzyme-linked immunosorbent assays (ELISA) are readily employed for clinical diagnosis. However, the sensitivity of these procedures is not high enough to detect the low number of viral particles sometimes present in the environment. In the case of environmental samples, amplification of viral nucleic acids by polymerase chain reaction (PCR) assays coupled to reverse transcription (RT-PCR) has been increasingly applied to detect a range of viruses in water and shellfish (Koopmans et al., 2001; Buesa et al., 2002; Bosch et al., 2004; Di Pinto et al., 2004).

ELISA is also routinely used for the detection of adenoviruses and astroviruses. Sapoviruses and noroviruses can be demonstrated using RT-PCR, or recombinant enzyme immune assay (rEIA) (Hutson et al., 2004). The results of study of Rabenau et al. (2003) showed that PCR has the highest sensitivity (94.1%), followed by transmission electron microscopy (TEM; 58.3%), and ELISA (31.3%) for detection of noroviruses in stool samples. However, specificity was highest for TEM (98.0%), followed by ELISA (94.9%), and PCR (92.4%). All three methods are, according to Rabenau et al. (2003), useful for epidemiological investigations in gastroenteritis outbreaks; however, to maximize diagnostic validity, at least two of the methods should be combined.

For screening for the presence of norovirus in shellfish and for human adenoviruses real-time-RT-nested PCR assay and nested PCR assay were used, respectively (Myrmel et al., 2004). Arenavirus-specific RNA can be detected in materials from patients using a nested RT-PCR assay. In addition, infectious arenavirus can be recovered from materials by cultivation of the virus in monolayer cultures of Vero E6 cells (Anonymous, 2000a).

Specific IgM antibodies, RT-PCR and real-time PCR methods can be employed for the detection of hepatitis A (Acha and Szyfres, 2003; Furuta et al., 2003; Hutson et al., 2004). By molecular biology-based methods, the most common foodborne viruses can be detected (*Norovirus*, hepatitis A and others) in shellfish, but none are usually available for other foods. The variability of the *Norovirus* genome hinders the development of a detection kit for routine use. A method to extract and detect human enteric viruses from food other than shellfish was reported by Leggitt and Jaykus (2000). This method used an elution – concentration approach followed by detection using RT-PCR. However, molecular diagnostic methods for food or water are not routinely available in food microbiology laboratories (Koopmans and Duizer, 2004).

4. Prevention of transmission of viral infections via food

Most documented foodborne viral outbreaks can be traced to food that has been manually handled by an infected food handler, rather than to industrially processed foods (Koopmans and Duizer, 2004). The viral contamination of food can occur anywhere in the process "from farm to fork", but most foodborne viral infections can be traced back to infected persons who handle food that is not heated or otherwise treated afterwards. Therefore, emphasis should be on stringent personal hygiene during preparation. If viruses are present in food pre-processing, residual viral infectivity may be present after some industrial processes. Other methods of inactivating viruses except adequate heating within a food are relatively unreliable, but viruses in water and on exposed surfaces can be inactivated with ultraviolet light or with strong oxidizing agents (Cliver, 1997a).

Therefore, it is key that sufficient attention should be given to good agriculture practice (GAP) and good manufacturing practice (GMP) to avoid introduction of viruses onto the raw material and into the food-manufacturing environment, and to HACCP to assure adequate control over viruses present during the manufacturing process. If viruses are present in foods after processing, they remain infectious in most circumstances and in most foods for several days or weeks, especially if kept cooled (at 4°C). According to Koopmans and Duizer (2004) it is necessary for the control of foodborne viral infections to:

(i) Heighten awareness about the presence and spread of these viruses by foodhandlers

(ii) Optimise and standardise methods for the detection of foodborne viruses (develop laboratory-based surveillance to detect large, common-source outbreaks at an early stage

(iii) Emphasise consideration of viruses in setting up food safety quality control and management systems (GHP, GMP, HACCP)

More strict and more frequent hygienie inspections of food manufacturers in all stages of production, processing, and distribution according to the hygienie requirements defined in Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs, should be made to ensure prevention of viral transmission via food, particularly in the post-communist, new member states of the European Union.

5. Conclusions

Despite the fact that viruses are the most common pathogens transmitted via food, for example causing 66.6% of food-related illnesses in the United States, compared to 9.7% and 14.2% for salmonella and campylobacter respectively (Mead et al., 1999), no systematic inspection and legislation exist that would set up virological criteria for food safety, regarding the presence of viruses in the food chain (Koopmans and Duizer, 2004). Accordingly, the education of food industry managers, producers, distributors, and consumers about hygienic regulations and conditions of food production and processing (the use of non-infected water for watering and food processing, clean utensils etc.) and particularly their compliance (Croci et al., 2002) are essential.

Acknowledgments

6. REFERENCES

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