Viruses as causative agents of foodborne diseases

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Abstract

Viruses (particularly noroviruses and hepatitis A virus) are important causes of growing numbers of foodborne diseases. They are highly resistant to environmental conditions and can persist for extended periods in the environment and in food. The principal sources of viral contamination of food include human or animal faeces and untreated sewage, infected food handlers and animals carrying zoonotic viruses (e.g. hepatitis E virus). Ready-to-eat food prepared by infected food handlers and bivalve molluscs often consumed raw or undercooked are categories of food particularly associated with viral foodborne illnesses. The detection of viruses in foodstuffs is usually based on molecular biological methods, though standardised methods of testing are not yet available. The regular monitoring of foodborne viruses is not required by law, but recommendations and procedures for the prevention of viral contamination are included in the Codex Alimentarius.

Foodborne infections, noroviruses, hepatitis A virus, hepatitis E virus, ready-to-eat foods, bivalve molluscs

Introduction

Viruses are non-cellular particles dependent on living cells for their replication. Unlike bacteria they cannot reproduce in food, and viral contamination has no effect on the organoleptic properties of food. A viral particle (a virion) is comprised of nucleic acid (deoxyribonucleic acid – DNA viruses, ribonucleic acid – RNA viruses) and a protein shell (a capsid). Some viruses also have an external membrane envelope. The size of viruses significant from the perspective of foodborne transmission ranges from 28 nm (the hepatitis A virus – HAV) to 100 nm (adenoviruses) (Carter 2005).

Viruses transmitted via food include non-enveloped human enteric viruses most frequently causing gastroenteritis (e.g. noroviruses – NoV, rotaviruses, sapoviruses etc.) and hepatitis (HAV and hepatitis E virus – HEV). The third group is comprised of viruses that reproduce in the gastrointestinal tract, but cause infection after migrating to the target organs, such as the central nervous system (e.g. the poliovirus) (Carter 2005; Newell et al. 2010).

Recently, the number of viral foodborne infections has been on the increase. According to the EFSA report, viral agents were the second most frequent cause of foodborne outbreaks in the EU (15%) following after salmonellas (30.5%) in 2010 (EFSA 2012). The most frequent viral agents were caliciviruses, including NoV. The proportions of various types of foodstuffs as sources of foodborne outbreaks caused by caliciviruses are given in Plate II, Fig. 1. The most frequent sites of the outbreaks were restaurants, hotels and bars (47.6% of cases) (EFSA 2012).

The numbers of cases of viral infections with possible transmission via foodstuffs in the Czech Republic are given in the EPIDAT system (Table 1). However, far larger numbers of viral enteric infections among the human population exist, as they are frequently not reported due to the mild symptoms and short course of the infection. The number of cases of hepatitis A was significantly higher in 2008 and 2009 when there was an outbreak of the disease. The disease spread quickly due to increased susceptibility among the general population, mainly through person-to-person contact. Transmission via foodstuffs was not confirmed in this outbreak (Částková and Beneš 2009). A gradual increase is being noted in the reported number of cases of hepatitis E. Hepatitis E used to be associated primarily

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with people travelling to endemic areas with low standards of hygiene. The increase of cases in recent years may be connected with changes in the eating habits of populations associated with the consumption of undercooked pork and pork products. However, a significant part in the increase is probably due to growing awareness of hepatitis E, searching for sources of the infection and thus higher screening in recent years.

Table 1. Numbers of cases of selected infectious diseases reported in the Czech Republic in 2002-2012 (EPIDAT)

Year	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Viral enteric infections	2381	2099	3590	3670	5597	6025	6639	6066	8517	9955	6870
Hepatitis A	127	114	70	322	132	128	1648	110	862	264	284
Hepatitis E	12	21	36	37	35	43	65	99	72	163	259

Foodborne viruses survive the low pH in the stomach, replicate in cells in the gastrointestinal tract and are excreted in large numbers (10^6 to 10^{11} particles g^{-1}) in faeces and/or vomit and saliva (FAO/WHO 2008). As few as 10 to 100 infectious virions are sufficient to cause an infection (EFSA 2011). Excretion of viral particles may occur in an infected person even if clinical symptoms have not yet appeared and even during asymptomatic infection (FAO/WHO 2008; Schmid et al. 2011). Besides, viral particles may continue to be excreted for several weeks depending on the type of virus – for as long as six months in the case of HAV (Koopmans and Duzier 2004).

Human enteric viruses are extremely infectious and person-to-person contact is, with certain exceptions, the most frequent route of their transmission. Foodstuffs also play a significant part in spreading viral infections, accounting for around 5% of cases of HAV and 12 - 47% of cases of NoV (FAO/WHO 2008). Viral foodborne gastroenteritis generally has a short incubation period (1 to 3 days) and its symptoms (vomiting, fever and diarrhoea) usually disappear without medical attention. The epidemiological data available do not provide adequate information on the occurrence of these diseases in the population, and the number of cases is evidently greatly underestimated.

Routes of viral contamination of foodstuffs

Contact between foodstuffs and viruses may occur at any stage of the processing, including the period before harvesting (fresh fruit, vegetables, live bivalve molluscs), during processing and during preparation for consumption. The three main sources of the contamination are human and animal faeces and untreated sewage, infected workers handling foodstuffs, and animals as a source of zoonotic viruses (FAO/WHO 2008; EFSA 2011). A combination of more than one route including interpersonal transmission is usually involved in spreading the viral agent in the cases of large outbreaks (Plate II, Fig. 2).

Animal viruses capable of causing disease in humans may enter the food chain via animal products from infected animals or through foods that have undergone secondary contamination during processing. Further transmission of viruses may also occur through person-to-person contact. These viruses include HEV, the highly pathogenic avian influenza (HPAI) virus, SARS-causing coronavirus and Nipah virus (FAO/WHO 2008).

The resistance of viruses to environmental conditions

Viruses transmitted by a faecal-oral route may remain infectious in contaminated food and the environment for a long time. The resistance of viruses may be increased by the presence of organic substances.

Refrigeration and freezing generally preserve viruses, and low temperatures are considered a significant factor supporting the persistence of foodborne viruses in the environment (Baert et al. 2009; Vasickova et al. 2010). UV radiation is effective in inactivating viruses, particularly on equipment surfaces, in water and in aerosols. The specific type of virus and the composition of the foodstuffs have, however, a profound influence (Vasickova et al. 2010; EFSA 2011). The effect of UV radiation and resistance at low temperatures affect the seasonal incidence of viral diseases, which occur primarily during the winter months.

Enteric viruses transmitted by the faecal-oral route are characterised by the resistance to a low pH that is important while they are passing through the gastrointestinal tract to sites of replication in the intestines or other organs. Although being most stable at a neutral pH, these viruses are more resistant to an acidic (3–5) than an alkaline (9–12) pH (Vasickova et al. 2010). Due to their resistance to a low pH, NoV and HAV are also likely to survive fermentation processes in the production of meat products (Baert et al. 2009).

Modified atmosphere packing does not have a significant effect on viral infectivity and is not, therefore, a suitable means of reducing viruses present in foodstuffs (Baert et al. 2009).

Resistance to heat treatment depends on the type of virus, the composition of food and the initial level of viral contamination (Codex Alimentarius 2012). The temperature of 70 °C for 2 minutes at the core of the food generally reduces infectious virions significantly, though it cannot ensure their complete inactivation (IFST 2008). Viral contamination in other foods occurs primarily on the surfaces where viruses are more sensitive to the effect of temperature.

The majority of the disinfectants (generally most commonly used alcoholic preparations) are not sufficiently effective to inactivate viruses (Duzier et al. 2004; EFSA 2011). Cleaning with hot water and detergent, followed by disinfection with a solution of 500 to 1000 ppm of free chlorine for 5 to 10 minutes is recommended as a suitable procedure (Codex Alimentarius 2012).

Foodstuffs representing a risk from the viewpoint of viral contamination

Foods intended for direct consumption, mainly fresh or only slightly processed fruit and vegetables and fast-food meals, are primarily associated with viral foodborne diseases (Fig. 1). High-risk foods include bivalve molluscs (oysters, clams and mussels) that are frequently consumed raw or undercooked. Their frequent viral contamination is associated with the ability to filter the water, which leads to the concentration of viral particles from the surrounding environment (Lees 2000). The classification of live bivalve molluscs production and harvesting areas (classes A to C) is based on the concentration of faecal indicator bacteria (*E. coli*) in the shellfish flesh and intravalvular liquid (Regulations (EC) 2073/2005 and 854/2004). Bivalve molluscs from class A areas are intended for direct human consumption, even though pathogenic viral species such as HAV, NoV and enteroviruses have been detected in them (Mesquita et al. 2011). In the majority of molluscs (classes B and C) depuration and relaying must be applied before they are sold on the market or must be cooked adequately with the aim of eliminating pathogenic microorganisms (Regulation (EC) 853/2004).

The depuration process takes place over a period of between one and seven days in tanks of clean seawater generally sterilised by ozone or UV radiation. The relaying comprises self-cleaning in a natural environment for at least 14 days. The given regulations, effective in eliminating bacterial pathogens differ, however, in terms of their effective reduction of viral contamination. In this respect, long-term relaying (for a minimum of two months) is considered sufficient, though it has distinct economic disadvantages and involves the problem of finding suitable areas of uncontaminated water (Lees 2000). Another option is a combination of depuration and relaying or

commercial heat treatment at a temperature of at least 90 °C for 90 seconds (Regulation (EC) 853/2004; IFST 2008). The production of molluscs in areas free of faecal contamination is, however, considered the most effective measure (EFSA 2011).

The outbreaks associated with the consumption of bivalve molluscs are usually caused by NoV and HAV, even though astroviruses, adenoviruses, rotaviruses, HEV and enteroviruses have also been frequently detected in the molluscs (Lees 2000; La Rosa et al. 2012). Bivalve molluscs are often contaminated with more than one type of virus, which may lead to new recombinant viruses with altered properties (Lees 2000).

In case of meat and meat products, attention is currently focused primarily on HEV due to its zoonotic potential. The target cells of the virus are hepatocytes; therefore the greatest risk is the consumption of liver and its products that have not been sufficiently heat treated. The genetic relationship between HEV isolates originating from retail pig liver and isolates from hepatitis E patients provides evidence for the existence of zoonotic transmission (Yazaki et al. 2003; Bouwknegt et al. 2007). Cases of hepatitis E have been described following the consumption of grilled pork and pig viscera (Miyashita et al. 2012), wild boar meat (Li et al. 2005; Masuda et al. 2005), raw deer meat (Tei et al. 2004), products made from raw pig liver fermented for a short period of time (Colson et al. 2010) and bivalve molluscs (Said et al. 2009). Numerous cases of HEV foodborne infection have been described in Japan where 2–3 tons of deer meat is consumed each year, often raw in the form of sashimi (Tei et al. 2004). Its consumption is a significant risk factor for infection in these areas.

Detection of foodborne viruses

The majority of foodborne viruses (NoV, HAV and HEV) cannot be cultured *in vitro*, or only with great difficulty. Their detection in foodstuffs is, therefore, generally based on molecular biological methods; detection of viral nucleic acids by PCR (DNA viruses) or and reverse transcription PCR (RNA viruses). However, these methods are not able to distinguish between infectious and non-infectious viral particles. The routine use of these methods is currently restricted to a small number of laboratories with the necessary equipment (EFSA 2011). The development of standardised and harmonised methods for the detection of NoV and HAV in foodstuffs is currently being completed under the auspices of the European Committee for Standardisation (CEN).

Diagnosis of viral diseases in humans is based on the detection of viruses or their nucleic acids in faecal samples, supplemented by epidemiological studies to uncover possible sources of the disease (Carter 2005; EFSA 2011). The ELISA method may also be used, either to determine the serum-specific antibodies (e.g. in patients with hepatitis A or E) or to detect the antigen in stool samples (e.g. NoV and rotaviruses), supplemented by electron microscopy, which is possible due to high concentration of virions in the sample (at least $10^6.g^{-1}$). Unfortunately, these methods are not sufficiently sensitive to find out viral contamination of foodstuffs with a low concentration of viral particles (Scipioni et al. 2008).

Systems monitoring the viral contamination of foodstuffs

Information on the viral contamination of foodstuffs appears in the European RASFF system (Rapid Alert System for Food and Feed), though this information is limited. The system most frequently reports the presence of NoV and HAV in live or frozen molluscs. Besides, the international network NoroNet allows virologists and other experts to share information on norovirus outbreaks and to predict them.

Legislative requirements for the monitoring of viral contamination in foodstuffs

To date, the valid legislation has not required the monitoring of viral agents in foodstuffs (Regulation (EC) 2073/2005). Only Regulation 1235/2012, which sets out the requirements

for the increased level of official controls on imports of certain feed and food of non-animal origin, indicates the risk of imports of strawberries from China in view of the presence of NoV and HAV. Criteria for the microbiological quality of foodstuffs are generally based on the detection of members of the family *Enterobacteriaceae* or *E. coli* as indicators of faecal contamination, poor production hygiene and poor quality of raw materials (Regulation (EC) 2073/2005). These criteria are, however, inadequate for the assessment and monitoring of viral contamination in foodstuffs (Newell et al. 2010; EFSA 2011).

The detection of bacteriophages (viruses infecting bacteria) present in large numbers in material contaminated with faeces can be used as an indicator of viral contamination (Lees 2000; Noble et al. 2003). Another possibility is to monitor other viruses (adenoviruses and enteroviruses) that are often present in human (animal) faeces and which are easier to detect than enteric viral pathogens (Noble et al. 2003).

Requirements for the hygiene of workers, means of sanitation and other procedures for preventing the viral contamination of food are included in the recommendations of the Codex Alimentarius (CAC/GL 79-2012; Codex Alimentarius 2012). The Codex Alimentarius is a collection of internationally recognised standards and recommendations related to food safety drawn up by the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO). Although these recommendations are not legally binding, they often represent a starting point in drawing up national and regional laws.

The most frequent viruses causing foodborne diseases

Hepatitis A virus

In the majority of developing countries where hepatitis A (an RNA virus of the genus *Hepatovirus*, family *Picornaviridae*) is an endemic disease, a large part of the population is infected in childhood when the infection is generally asymptomatic. In developed countries, HAV infection is less common due to higher hygienic standards and the availability of vaccination. The majority of the adult population therefore remains susceptible, and the potential risk of a hepatitis A outbreaks increases. The excretion of viral particles begins 10 to 14 days before clinical symptoms and may continue for as long as six months (Koopmans and Duzier 2004). Infected workers may contaminate foodstuffs during handling if hygiene rules are not observed. However, it has been discovered that merely washing hands with running water statistically significantly reduces the number of HAV particles (Bidawid et al. 2000). In addition to fresh fruit and vegetables (in particular soft fruit, lettuce and green onions), hepatitis A is also associated with the consumption of insufficiently cooked molluscs (Lees 2000; Mesquita et al. 2011). An outbreak of hepatitis A (269 cases of the disease in Belgium) evidently caused by the consumption of raw beef contaminated by an infected worker during distribution has also been described (Robesyn et al. 2009). The long incubation period of the disease (3 to 6 weeks) does not allow to trace contaminated food and to determine the source of infection (Koopmans and Duzier 2004). Vaccination of workers is particularly recommended in endemic areas and in case of low immunity of the population (Codex Alimentarius 2012).

Noroviruses and sapoviruses (SaV)

Norovirus and *Sapovirus* are genera of non-enveloped viruses with single-stranded RNA belonging to the family *Caliciviridae*. NoVs are the most frequent cause of non-bacterial gastroenteritis in humans; SaVs play a less significant role, particularly as agents of gastroenteritis in children (Bank-Wolf et al. 2010). NoVs can be divided into five genogroups (G). Viruses belonging to genogroups GI, GII and GIV cause infection in humans; GIII has been detected in cattle and GV in mice (Scipioni et al. 2008). Viruses related to genotype GII.4, which most commonly causes illness in people, have also been detected in the faeces of pigs and cattle and in a sample of retail pork meat

(Mattison et al. 2007; Nakamura et al. 2010). The results of these studies indicate possible zoonotic transmission of NoV. NoVs are highly infectious, but the illness is relatively mild (diarrhoea, vomiting, stomach ache) and lasts 12 to 72 hours. However, NoV may cause more serious illness in the elderly and people with underlying disease (Koopmans and Duizer 2004). No vaccine against NoV is yet available.

SaV can be divided into genogroups GI to GV, of which GI, GII, GIV and GV have been detected in people and GIII in pigs. Genotypes of which many show a genetic relationship with human SaV have also been described in pigs, which indicates the possibility of zoonotic transmission as well (Nakamura et al. 2010).

Rotaviruses

Rotaviruses (Plate III, Fig. 3) are non-enveloped RNA viruses belonging to the family *Reoviridae*. They can be divided into seven serogroups. Rotaviruses of groups A to C cause illness in humans and other mammals, including livestock. Groups D, F and G occur in poultry, while group E has been detected in pigs (Martela et al. 2010). Rotaviruses, particularly group A, are the principal cause of viral gastroenteritis in children and also cause serious illness associated with dehydration. The main transmission route is person-to-person contact, though their transmission by water and food also plays an important role (Carter 2005).

Hepatitis E virus

HEV is a non-enveloped RNA virus and the only member of the genus *Hepevirus* belonging to the family *Hepeviridae* (Meng 2011). Genotypes 1 and 2 of the virus occur primarily in developing countries in which the occurrence of hepatitis E is endemic and where it spreads principally via faecal contamination of water. In developed countries, hepatitis E used to be associated with travel to endemic areas, though cases associated with the consumption of insufficiently heat treated meat and organs from domestic pigs, wild boars and deer appear frequently (Tei et al. 2004; Li et al. 2005; Miyashita et al. 2012). These cases are primarily caused by genotypes 3 and 4, which also occur in animals (Meng 2011). The clinical symptoms in humans (nausea, lethargy, fever, jaundice) are comparable to hepatitis, but the incubation period is usually longer (40 days on average). The disease is not generally fatal and is often asymptomatic. A higher mortality rate (even exceeding 20%) is described only in pregnant women (Miyashita et al. 2012). Domestic pigs (Plate III, Fig. 4), wild boars and cervids are without clinical signs of disease and currently are considered as reservoir of HEV (Leblanc et al. 2007; Meng 2011). Veterinary inspection does not reveal infected animals and the virus may be present in meat and organs entering the food chain (Feagins et al. 2007; Di Bartolo et al. 2012).

Pigs become infected most often between 2 and 4 months old through direct contact with infected animals or by contaminated water and feed (Meng 2011). The majority of animals have HEV antibodies at the time of slaughter, though the excretion of the virus in faeces is less frequent. Replication of HEV also occurs in the lymph nodes and in the small and large intestinal tissue, in addition to the liver (Williams et al. 2001). In this respect, there would seem to be a risk involved in the use of pig intestine as casing material for meat products. Table 2 shows the detection of HEV in samples of pig liver intended for human consumption. Viral RNA was detected in the liver in the studies mentioned, though this does not necessarily mean that infectious viral particles were present. Storage conditions during distribution (4 or –18 °C) may also inactivate the virus (Feagins et al. 2007).

According to certain studies (Emerson et al. 2005; Feagins et al. 2008), a temperature of 70 °C or 95 °C for 10 minutes completely inactivates the virus, though it remains infectious at 56 °C. In an *in vivo* study in pigs, Barnaud et al. (2012), however, discovered that

Table 2. Detection of HEV RNA in pig liver intended for consumption in various countries

No of liver samples tested (site of collection)	No of positive samples (%)	Country	Reference
363 (retail)	7 (1.9)	Japan	Yazaki et al. 2003
127 (retail)	14 (11.0)	USA	Feagins et al. 2007
62 (retail)	4 (6.5)	Netherlands	Bouwknegt et al. 2007
240 (retail)	2 (0.8)	India	Kulkarni and Arankalle 2008
40 (slaughter)	5 (5.0)	Czech Republic	Di Bartolo et al. 2012
33 (slaughter)	2 (6.0)	Italy	Di Bartolo et al. 2012
39 (slaughter)	1 (3.0)	Spain	Di Bartolo et al. 2012

only heat treatment at 70 °C for 20 minutes is able to inactivate infectious viral particles. Therefore heat treatment, understood as the attainment of a thermal effect corresponding to the action of a temperature of 70 °C for a period of 10 minutes in all parts of the product (Decree No. 326/2001 Coll.), appears to be insufficient for the complete inactivation of HEV.

The situation in the Czech Republic

In the Czech Republic, the presence of HEV genome was discovered in at least one out of 10 piglets (71.4%) on 14 farms monitored (Kosinová et al. 2012). HEV RNA was detected in at least one sample (bile or intestinal content) from 27 (42.8%) of 63 piglets tested. The results of the study by Di Bartolo et al. (2012) show that HEV RNA was found in 3 (8%) out of 40 healthy pigs slaughtered in the Czech Republic, and the findings were in samples of faeces (3%), liver (5%) or tongue muscle (3%). None of the 92 retail meat products (sausages) tested contained HEV genome.

HEV isolates of genotypes 3f and 3g genetically related to Czech isolates from pigs were obtained from clinical samples from patients in the Czech Republic, which indicates possible zoonotic transmission of the virus (Vasickova et al. 2011). These patients also reported consumption of homemade pork specialities and the use of the same knife and other tools for raw and cooked pork. This indicates possible cross-contamination during the preparation and cooking of meat. The increase in the number of cases of hepatitis E recorded in the Czech Republic in recent years is given in Table 1.

Other viruses that may be transmitted by food

Faecal-oral dissemination is also a possible route of transmission of certain primarily respiratory viral pathogens. Transmission of the Nipah virus by fruit contaminated with urine or saliva of bats of the genus *Pteropus*, which is considered a natural reservoir of the virus, has been described (Luby et al. 2009). Detection of the dangerous subtype H5N1 of the HPAI virus in duck meat suggests the possible role of the food chain in spreading this virus (Tumpey et al. 2002). This virus is, however, inactivated by sufficient heat treatment (70 °C for at least 1 second) (Swayne 2006). Transmission by contaminated food cannot be excluded in the case of the SARS-causing coronavirus (McKinney et al. 2006).

Adenoviruses (Plate xy, Fig. 5) are other primarily respiratory viruses, though they can also replicate in the digestive tract and be present in faeces. Out of 51 serotypes, types 40 and 41 cause gastroenteritis, particularly in children under the age of five years (Carter 2005). Adenoviruses are considered significant faecal and/or viral pollution indicators in the environment and water (Pina et al. 1998).

The study by Chu et al. (2012) described the presence of gyroviruses (non-enveloped

DNA viruses of the family *Circoviridae*) in the faeces of patients with diarrhoea, respiratory or feverish illnesses, and in chicken meat and skin. It is clear that members of the gyroviruses, which include the chicken anaemia virus, get into the human body via consumption of chicken meat. Further study is required to determine whether gyroviruses are capable of replication in the human gastrointestinal tract or if they are only passively excreted in the faeces.

Consumption of unpasteurised goat, sheep and cow milk and their derivatives is a possible route of the transmission of the tick-borne encephalitis virus (the family *Flaviviridae*) to humans (Dobler et al. 2012).

Conclusions

Great attention is devoted to foodborne viruses, their sources and routes of dissemination as can be seen from the reports and viewpoints of organisations active in the area of food safety (FAO/WHO 2008; EFSA 2011, 2012; Codex Alimentarius 2012). The introduction of standardised methods of detecting viruses in foodstuffs is considered to be of fundamental importance, and will help gather the necessary information about sources and routes of viral contamination of food. Control mechanisms should be primarily focused on preventing food contamination. The availability of appropriate means of hygiene, along with instructions relating to the correct washing and disinfecting of hands and contaminated surfaces, is also important. These measures should be incorporated into the existing HACCP systems and other control mechanisms in food processing plants.

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Plate II
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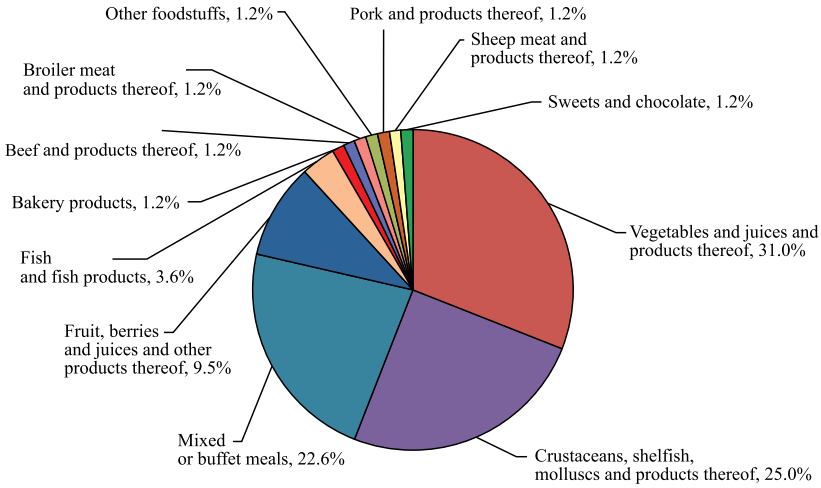

Fig. 1. Foodstuffs responsible for outbreaks caused by caliciviruses (N = 84) in the EU in 2010 (EFSA 2012)

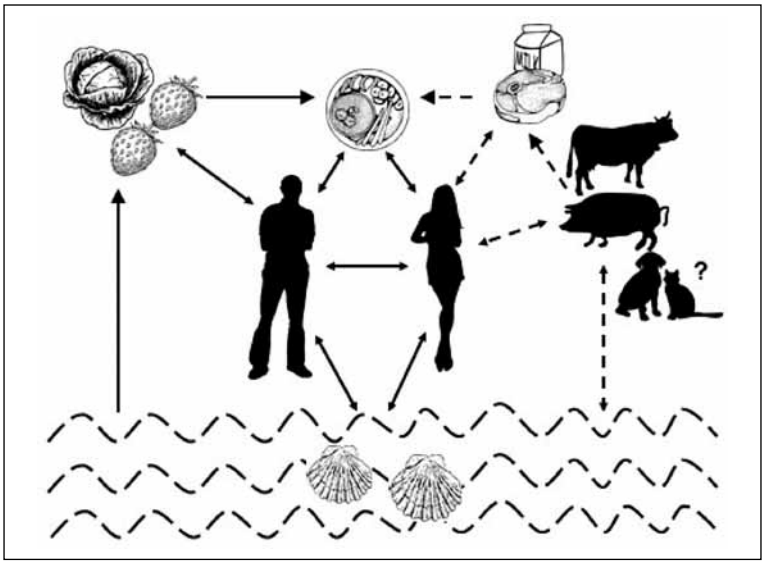

Fig. 2. Sources and routes of transmission of foodborne viruses. Discontinuous lines indicate possible transmission of zoonotic viruses (Vasickova P)

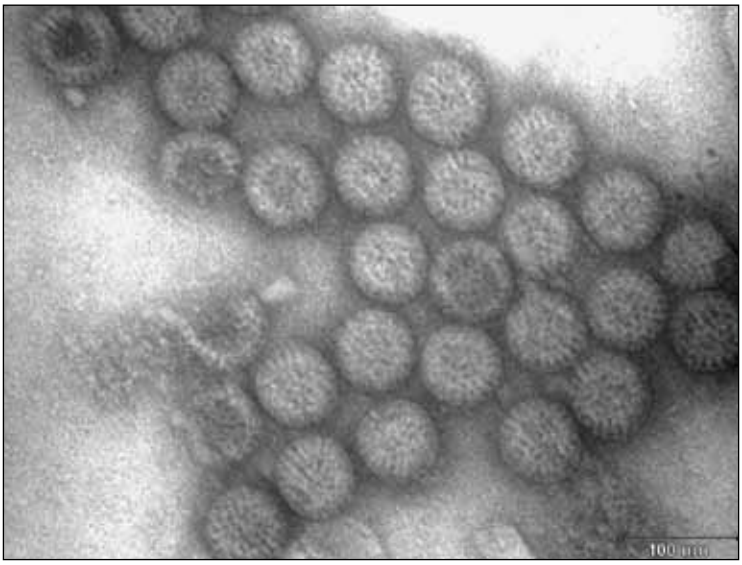

Fig. 3. Rotavirus, calf faeces - electron microscope (Kulich P)

Fig. 4. Domestic pigs are reservoir of hepatitis E virus (Trckova M)

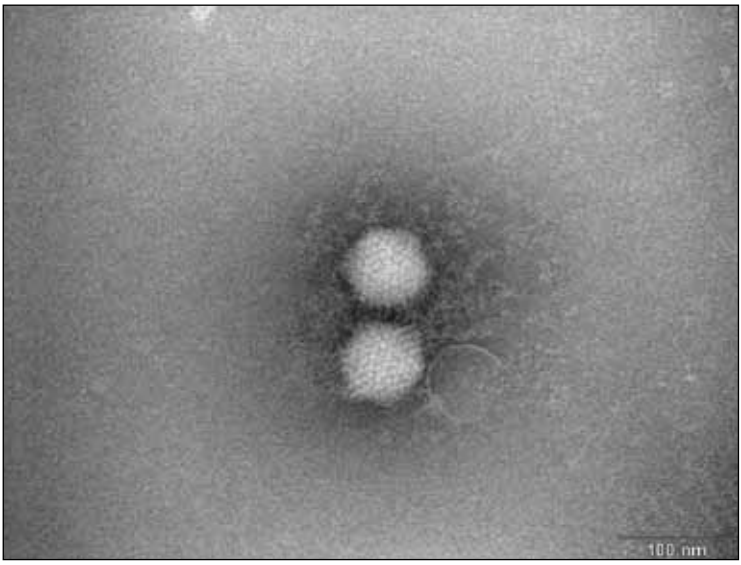

Fig. 5. Adenovirus, piglet - electron microscope (Kulich P)