FOOD-BORNE PATHOGENS

Enteric virus contamination of foods through industrial practices: a primer on intervention strategies

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Hepatitis A and E viruses, rotaviruses, Norwalk-like caliciviruses, and astroviruses are among the enteric viruses known to cause food- and waterborne illness. These viruses are spread by the fecal–oral route and are a major cause of morbidity and mortality worldwide. Foods may be contaminated at any time pre- or post-harvest; however, many outbreaks are associated with foods handled by infected restaurant workers. Produce may be contaminated by improper irrigation or fertilization practices, by the hands of infected pickers or processors, or as the result of adulteration during any stage of handling. Outbreaks have been commonly associated with foods which are served raw or only lightly cooked, such as molluscan shellfish, fruits and vegetables, and salads or products contaminated after cooking like frosted bakery products. The farming, shellfish, processing, transportation, and restaurant industries must maintain vigilance to reduce outbreaks of enteric virus illness. Intervention strategies to enhance product safety include increased industry and consumer education; changes in industrial practices, product management, and processing technologies; worker immunizations; and the development of improved monitoring tools for the detection of enteric viruses in foods. Journal of Industrial Microbiology & Biotechnology (2001) 27, 117–125.

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Introduction

Enteric viruses comprise a broad assortment of viruses that are transmissible to humans through contaminated food and water and through person-to-person contamination. Among the most notable foodborne enteric viruses are hepatitis A and E viruses, small round-structured viruses, and group A rotaviruses. All of these viruses can be spread readily to foods during food production, processing, and handling. Hepatitis A virus has contributed to numerous foodborne outbreaks and is often associated with raw or lightly cooked shellfish [83]. The largest foodborne outbreak of hepatitis A was from the consumption of contaminated clams where nearly 300,000 people became sick during a 3-month period [38]. In the United States, hepatitis A causes an estimated 83,000 illnesses per year [70]. To date, there have been only two reported cases of domestically acquired hepatitis E in the United States [29,56]. Epidemics of hepatitis E have been reported in Asia, Africa, and Mexico [19]. Both of these viruses can migrate from the intestinal tract to infect and damage the liver, leading to jaundice and, occasionally, death. Hepatitis A is often self-limiting and spontaneous recovery is common [88]. The fatality rate from hepatitis A is about 100 per year in the United States [57]. Hepatitis E has a low mortality rate for the general population (0.5–3.0%); however, pregnant women have a mortality rate between 15% and 25% [64]. The incubation period for hepatitis A is 25–45 days, whereas the incubation period for hepatitis E is 15–60 days.

Caliciviruses, small round-structured viruses, may be divided into three genogroups represented by Norwalk virus, Snow Mountain virus, and Sapporo virus. Genogroup I viruses include Norwalk, Southampton, and Desert Shield viruses. Genogroup II includes the Snow Mountain, Camberwell, Lordsdale, Mexico, Toronto, Bristol, Hawaii, and OTH-25 viruses. Collectively, genogroups I and II viruses may be referred to as Norwalk-like viruses. Genogroup III consists of the classical caliciviruses with Sapporo virus as the prototype. Other members of this genogroup include Houston, London, and Parkville viruses. The Centers for Disease Control and Prevention recently estimated the annual incidence of Norwalk-like viral illness in the United States at 23 million [70]. The caliciviruses may cause diarrhea and vomiting with rapid onset, but lasting for only a few days. Norwalk-like viruses have been listed as the leading cause of foodborne hospitalizations in the United States today and responsible for 7% of the food-related deaths [70].

Rotavirus illness, also referred to as acute infantile diarrhea, is a common cause of infantile diarrhea worldwide. The exact role of foods in rotavirus transmission has not been elucidated; however, food, water, and person-to-person are all vehicles of transmission. It was estimated that only 1% of rotavirus cases was foodborne [70]. Rotavirus diarrhea may lead to dehydration and death, particularly in regions of the world where rehydration therapy is not available. Rotavirus causes an estimated 2.7–3.9 million illnesses, 49,000–50,000 hospitalizations [9,70], and about 30 deaths per year in the United States [70], but an estimated 800,000 deaths per year worldwide [76]. Although rare in the United States, rotavirus deaths are common in poor countries where rehydration therapy is unavailable.

Other enteric viruses include the astroviruses, enteric adenoviruses, and coronaviruses, which are also spread by the fecal–oral route. The number of astrovirus infections in the United States is estimated at 3.9 million per year with only 10 deaths [70]. Water
and person-to-person transmissions may be responsible for the majority of illnesses from hepatitis A, rotavirus, and astroviruses; but 40% (about 9.2 million cases) of Norwalk-like virus illnesses may be foodborne [70]. The number of enteric virus illnesses in the United States can only be estimated because most of the illnesses are mild, go unreported, and routine testing of patients for specific virus infections is not performed.

Unlike some bacterial pathogens which may require a moderate to high dose to induce illness, it is believed that just a few virus particles may be sufficient to elicit infection. Viral contaminants may persist in the environment, on food surfaces, or within foods for extended periods [25,94,96]. Hepatitis A virus was detected in cream cookies maintained at 21°C and 49°C for up to 30 and 14 days, respectively, in oysters maintained at 12–24°C for >5 days and in environmental samples (soil and water) at 25°C for 56–84 days [94]. Fortunately, human enteric viruses can only replicate in susceptible mammalian hosts, so there is no virus amplification within foods.

Enteroviruses consist of poliovirus, echovirus, coxsackievirus types A and B, and enterovirus serotypes 70 and 71. Poliovirus has been used extensively as a laboratory model since it is similar to hepatitis A virus in some respects, but unlike hepatitis A virus, it can be readily propagated in cell culture. The use of poliovirus has provided much information on the effectiveness of various treatments and processes for the disinfection of foods and food contact surfaces. Nearly all of the polioviruses present in the environment are vaccine strains which enter the environment from the stools of children recently immunized with an attenuated oral poliovirus vaccine. This vaccine is being phased out and the vaccine strains will have to be destroyed. Soon, the poliovirus model will be a thing of the past. Unfortunately, there is no good successor for poliovirus; however, feline calcivirus is becoming a popular model for research on Norwalk-like viruses [93].

Pre-harvest contamination

Determination of the source of contamination is fundamental to development of an effective remedy. Pre-harvest contamination may occur in foods subjected to irrigation with reclaimed wastewater, crop fertilization with sewage sludge, or fecal pollution of the areas in which food products are obtained [14,75,90]. Non-point sources of pollution, like land runoff into watersheds and discharge of human wastes from boats, have caused major pollution events leading to foodborne illness [8,13,54,67]. Improperly operated sewage treatment plants, plants overloaded with storm water, or damaged or poorly maintained septic systems have been linked to outbreaks of enteric virus illness [73,99]. Land disposal of sewage sludge may contaminate groundwater and lead to potential disease outbreaks [96]. Improper dumping of septage or the leaching from baby diaper and related wastes from sanitary landfills can contribute to contamination of groundwater, which may in turn be used for drinking or irrigation. Dumps also contain insects, rodents, birds, raccoons, and other pests which may carry viruses many miles from the original disposal site. It is uncertain as to what extent wind and evaporation contribute to virus dispersal, although the incidence of illness among farm workers using spray irrigation of wastewater appears low [33,59]. During harvest, foods may be contaminated by harvesters, as was suspected in outbreaks of hepatitis A from strawberries [7,46,74], raspberries [82], and lettuce [89]; rotavirus from lettuce [41]; and Norwalk-like illness from raspberries [35] and oysters [54,67].

Post-harvest contamination

Enteric virus outbreaks have been associated with post-harvest contamination from the hands of food handlers, recontamination after cooking or processing, and inadequate sanitation. The restaurant industry has contributed to the incidence of illness due to poor managerial practice. Infected workers handling foods may transmit hepatitis A virus, Norwalk-like virus, and other enteric viruses to foods and no level of food processing will eliminate post-processing contamination. Virtually any food handled by ill workers may become contaminated, although certain food products have received more attention than others. Among the foods most commonly cited as contributing to viral illness from handling by infected workers are salads, raw fruits and vegetables [36,52,61,62,78], and bakery products [103,107]—foods that may be handled frequently and are not generally heated or reheated after handling. Cross-contamination of processed products by raw materials, like the contamination of salad by raw seafood, has also been reported [36]. Adequate plant sanitation may reduce the threat of product cross-contamination. Water must be of sanitary quality if it is to be used in food processing as an added ingredient in food preparation, or for use in manufacturing ice. Celery contaminated with non-potable water was responsible for an outbreak of Norwalk virus gastroenteritis, causing morbidity in over 1400 people [105]. Ice may become contaminated during or after production and can lead to food- or water-borne illness [12,51,58].

Industries at risk

Although most food-related industries are conscientious in producing safe and wholesome products, some are at higher risk of producing tainted products by the very nature of the food or the production process. Foods served raw or only lightly cooked are at greatest risk of transmitting viral contaminants obtained from either pre- or post-harvest sources. The molluscan shellfish industry is particularly susceptible since: (i) shellfish can bioconcentrate viruses within their edible tissues to levels much higher than in the water itself; (ii) there are no good methods to ascertain whether the shellfish or their harvest waters contain infectious viruses; and (iii) shellfish are frequently eaten raw or only lightly cooked. Outbreaks of foodborne viral illness have been attributable to virus-contaminated shellfish [20,83]. For the fruit and vegetable industries, farming practices, including fertilization and irrigation techniques, product transportation, and the health of the harvesters themselves, are coming under increased scrutiny. Today, food processors have an extensive list of disinfectants for sanitizing processing surfaces, but many disinfectants are ineffective in inactivating enteric viruses. Processors may also be unfamiliar with the best methods to apply disinfectants. There are many questions concerning processing conditions necessary to fully eliminate virus contaminants and further research is needed in this area. The
transportation industry must share the responsibility of maintaining clean trucks, ice, boxes, pallets, etc., to haul food products. It is fortunate for food transporters that human enteric viruses do not replicate in foods even under abuse temperatures. The transportation industry should follow the same safeguards as for protecting foods from external sources of bacterial contamination. Perhaps, the segment of the economy most at risk from virus contamination of foods is the restaurant industry. They are impacted most because they have a greater potential for contaminating foods due to extensive handling, often by inexperienced and untrained workers. Restaurants may be held liable in civil and criminal prosecutions if their products cause illness. The restaurant trade is, by no means, the only industry culpable in the event of an outbreak, so all facets of the production from the growth, harvest, and distribution, to the processing and marketing industries must be accountable for their role in ensuring safe foods. Hotels and restaurants reportedly have a high level of virus transmission through person-to-person contacts [68]. Cruise ship lines have also been hit hard by enteric virus-like illness [42,43,51,69]. Outbreaks often involve a substantial number of passengers and crew. In these cases, transmission is not only from direct consumption of contaminated foods, but likely from person-to-person spread.

**Intervention strategies**

There are certain fundamental industrial practices that will allow the elimination or significant reduction of viruses from foods. Methods for elimination of enteric viruses from foods depend somewhat on the specific virus present and the food contaminated. No one step will be sufficient to ensure a safe and wholesome product since recontamination may occur at any stage in the food chain; however, a concerted effort will reduce the risk of foodborne illness. The following are some strategies that the food industry may consider adopting.

**Industrial practices**

The practice of using wastewater for crop irrigation and sewage sludge for fertilization should be evaluated carefully. One study demonstrated the ineffectiveness of anaerobic mesophilic digestion at 35–36°C to inactivate rotavirus and coxsackie B5 viruses; however, anaerobic thermophilic digestion at 54–56°C and aerobic thermophilic fermentation at 60–61°C inactivated these viruses [95]. Wastewater should be subjected to, at least, primary and secondary treatments followed by chlorination or ultraviolet (UV) light disinfection to reduce virus levels. Wastewater irrigation may pose a particular risk for produce that is consumed raw. Viruses from spray irrigation may be harbored on the surfaces of fruits and vegetables and can lead to illness if the produce is not adequately washed or peeled. An antibacterial compound for use in washing and disinfecting the surfaces of fruits and vegetables was recently marketed and could offer some benefits in reducing the levels of external viral contamination.

The harvest of shellfish should be strictly regulated in regard to the criteria of the National Shellfish Sanitation Program and the water quality criteria set forth by the Interstate Shellfish Sanitation Conference [100]. Under this program, shellfish-growing waters are classified based on the levels of coliform bacteria in the water. Although the fecal coliform standard does not correlate directly with the levels of viruses in the water or in the shellfish, outbreaks of enteric virus illness are not often reported for shellfish obtained from approved harvesting waters. The shellfish industry and regulatory agencies should continue to actively pursue shellfish poachers who obtain shellstock from closed areas. Sacks of shellfish should be clearly marked with tags providing information required by state health authorities regarding the date and location of the harvest and the name of the harvester. Such information is crucial in epidemiological studies designed to track and remove from the market foods implicated in outbreaks.

Sick food handlers have been responsible for numerous cases of foodborne viral illness [24,78,79,108]. All food industries should mandate that sick workers do not handle foods, packaging materials or ingredients, or processing line equipment, dishes or utensils during or immediately after their illness. Workers who have seemingly recovered from an illness may still carry and shed viruses which can lead to food contamination [108]. Employers should require that sick workers not be allowed to work until they are better, and the employees should be given temporary jobs away from the food for a few days after recovering from enteric illness. Provisions should be made to financially help sick employees, through paid sick leave or by other means, so that the workers will not hide the fact that they are sick for fear of a reduced paycheck.

**Product and process management**

Viruses may be present in seemingly clean foods. Since viruses are not associated with product spoilage, flavor and odor changes that signal spoilage from bacteria will not indicate the virological quality or safety of foods. Therefore, it is important to provide a system of checks and balances to ensure the safety of all foods. The food industry has subscribed to a new approach to protect the nation’s food supply known as the Hazard Analysis Critical Control Point (HACCP) system [10,16]. Under the HACCP system, food operations must develop and implement a plan, based on sound scientific data and reasoning, to identify those points in the operation most likely to contribute to product contamination. These become the critical control points for the operation. The HACCP approach also requires that the critical control points be monitored to ensure proper operation of the system. In the case of cooked products, cooking times and temperatures would be monitored to ensure adequate inactivation of microbes. For canning operations, times and temperatures are routinely monitored to ensure that they are adequate to eliminate the presence of *Clostridium* spores. Similar safeguards are needed to control and eliminate viral contaminants. Many factors go into the development of a HACCP plan, but all too often, the health status of the employee is highly subjective. To reduce foodborne illness, employee health must be a prime consideration for all sectors of the food industry. Epidemiological data should also be used to design HACCP and risk assessment systems [81]. Over the next few years, HACCP is likely to expand into new areas of the food industry to encompass all aspects of the farm-to-fork continuum.

One fundamental attribute of the HACCP system is the monitoring aspect, where data and records are maintained and scrutinized for various critical control points. Under many HACCP programs, bacteriological tests are required to ensure that adequate processing times and temperatures are maintained or to demonstrate adequate sanitation of the equipment. Unfortunately, routine testing of viral contaminants in foods is not possible because virus assay procedures are generally lacking. In the absence of sound methods for virus detection, it may be necessary to identify potential hazards...
not empirically identifiable. As improved detection methods become available for viruses, critical control points for viral contaminants may be identified.

Processing technology
Industrial application of processing technologies to reduce or eliminate viral contamination from foods can be highly effective, depending on the food type and the technology applied. Some of the processes that are effective in preventing, reducing, or eliminating enteric viruses in foods are discussed below.

Food disinfection practices

Disinfection and sanitation
Good sanitation can reduce the incidence of foodborne contamination. Sanitation guidelines should be in place for foods from production and harvest to the consumer. Current HACCP systems are beneficial in identifying and controlling those factors critical to the maintenance of safe products.

A number of studies determined the effectiveness of disinfectants in eliminating enteric viruses (Table 1). References from Table 1 should be scrutinized closely to determine if the disinfectants are appropriate for specific applications. Chemical disinfectants should be selected based on their effectiveness against foodborne viruses. Although solutions containing chlorine are effective disinfectants for most viruses, other disinfectants may be more practical or have residual effects to enhance sanitation efforts. In one study evaluating 27 chemical disinfectants for their ability to inactivate rotavirus on glass, plastic, and stainless steel surfaces, only nine were effective: chloramine-T, chlorhexidine gluconate, glutaraldehyde, hydrochloric acid, isopropyl alcohol, peracetic acid, povidone iodine, quaternary ammonium compound, and sodium-o-benzyl-p-chlorophenate [60]. The treatment of Norwalk virus with up to 10 mg/ml of chlorine for 30 min was insufficient to inactivate all the viruses present as determined by a volunteer study [50], although there is some speculation that the amount of free chlorine available was substantially less than 10 mg/ml. In another study, chlorine, chloramine-dioxide, peracetic acid, and ozone were effective in reducing poliovirus, echovirus, coxsackievirus type B5, and rotavirus [39]. Rotavirus was readily inactivated by chlorine dioxide at alkaline pH values [22] and by chlorine [102] and ozone [101], particularly at acidic and neutral pH values. Chlorine dioxide and iodine, at alkaline pH values, have also been effective in inactivating poliovirus [3]. Engelbrecht et al [28] demonstrated that poliovirus types 1 and 2, echovirus types 1 and 5, and coxsackievirus types A9 and B5 were 99% inactivated in 0.3–4.5 min with residual chlorine levels of ~0.5 mg/l at pH values of 6.0 and 7.8; however, at pH 10, inactivation times were substantially increased (21–96 min) for all viruses except coxsackie A9, which was still rapidly inactivated after 1.5 min. Chlorine solutions at low pH (pH 6) were previously shown to inactivate enteric viruses more readily than chlorine solutions maintained at pH 10 [28]. Poliovirus was treated with 0.5 mg/l of free chlorine for various intervals, and virus propagation was attempted in cell culture followed by detection by reverse transcription polymerase chain reaction (RT-PCR) [17]. Poliovirus required 10 min for inactivation to non-detectable levels, demonstrating that poliovirus is five times more sensitive than previously expected [17].

Sattar et al [91] evaluated a host of potential disinfectants for the inactivation of rotaviruses and found that the best inactivation occurred with a 3-min exposure to a spray containing o-phenylphenol and ethanol; however, chlorine bleach and a phenolic compound were also effective in eliminating the viruses over a longer exposure period. A quaternary ammonium-based product was ineffective as a disinfectant [91].

Depending on the food type, chemical disinfection of viruses from surfaces of foods may be difficult or impossible to accomplish. Disinfectant solutions or detergents incorporated into the rinse water may facilitate the elimination of gross external contamination, but may be insufficient to remove viral contaminants, particularly if the viruses have penetrated through the skin. Washing and disinfection may not be effective for cut vegetables since viruses may enter the tissues through cuts and abrasions. Leafy vegetables may be more difficult to disinfect because of the rough or wrinkled nature of the surfaces. Likewise, fruits such as strawberries, raspberries, and blackberries have a porous surface texture which may trap viruses beyond the reach of chemical disinfectants. Rinsing without the use of suitable detergents or disinfectants may allow viruses to infiltrate the tissues of fruits and vegetables and to become redistributed onto products that were not previously contaminated. Therefore,

| Enteric virus | Substrate | Reference |
|--------------|-----------|-----------|
| Coxsackie B3, adenovirus, coronavirus | Organic suspensions on stainless steel surface | Sattar et al [92] |
| Coxsackie B3 and B5, poliovirus | Buffers | Jensen et al [48] |
| Poliovirus | Water | Blackmer et al [17] |
| Poliovirus | Blood and saline solution | Weber et al [106] |
| Poliovirus | Buffers | Alvarez and O’Brien [3] |
| Hepatitis A virus | Stainless steel surface | Mbithi et al [65] |
| Hepatitis A and rotavirus | Polystyrene surface, fomites, suspensions | Abad et al [2] |
| Rotavirus | Steel, glass and plastic surfaces, instrument soaks | Lloyd-Evans et al [60] |
| Rotavirus | Stainless steel surface | Sattar et al [91] |
| Rotavirus | Water and dried organic suspensions (feces, media, milk) | Ward et al [104] |
| Rotavirus | Buffer | Chen and Vaughn [22]; Vaughn et al [101] |
| Feline calicivirus | Suspending and dried preparations | Doughtrey et al [27] |
| Norwalk virus | Drinking water | Keswick et al [50] |
| Six enteroviruses | Buffer | Engelbrecht et al [28] |
individual washing of produce would be better than bulk washing to prevent cross-contamination. Potential disinfectants and cleaning agents must be scrutinized carefully to ensure that they are approved for use in food establishments or on the foods themselves.

Sanitation of equipment and food contact surfaces should employ the use of ample detergents and water to rinse away and dilute — if nothing else — the viruses associated with food residues or hand contamination. Large-scale food processing plants should consider the use of commercial sanitation stations utilizing hot disinfectant solutions and high-pressure water rinses for inactivation of microorganisms and removal of food residues from processing equipment and food contact surfaces.

Cooking/heating
Thorough cooking is one of the best and most practical methods to totally inactivate enteric viruses; however, the times and temperatures required vary with the composition of the food. High protein and fat content tends to enhance thermal stability of enteric viruses [34]. Enteric viruses were inactivated from hamburgers heated to an internal temperature of 60°C (rare), but only when the patties were rapidly cooled after cooking [97]. Studies performed by adding $1.0 - 1.9 \times 10^8$ poliovirus per gram of oysters followed by stewing, frying, baking, and steaming gave varying results, depending on the cooking method [26]. Thirteen percent of the viruses remained infective in fried oysters cooked to an internal temperature of 100°C (requiring 8 min); whereas baking to 90°C (requiring 29 min), stewing to 97.3°C (requiring 30 min), and stewing to 75°C (requiring 8 min) left 12.7%, 7%, and 10% of the viruses remaining, respectively [26]. Another gastroenteritis virus, the astrovirus, was inactivated by heating to 60°C for 10 min [55]. Studies on heat inactivation of hepatitis A virus in skim milk, homogenized milk, and table cream demonstrated a 5-log reduction with exposure of 85°C for <0.5 min; whereas at 80°C, skim and homogenized milk required up to 0.68 min and cream required 1.24 min for similar reductions [15]. Their findings [15] supported the conclusion that higher fat content provides thermal stability for hepatitis A virus.

Irradiation
Irradiation, UV light, and microwave energy are all effective in reducing enteric viruses in foods. Viral nucleic acids are fragmented in the presence of ionizing radiation. Irradiating radiation, in the form of gamma emissions, is becoming more generally accepted for food processing [4,31,32]. Depending on the product, irradiation levels as high as 10 kGy may be used. A petition currently before the U.S. Food and Drug Administration seeks to allow the use of 4.5 kGy for non-frozen and non-dry products and 10.0 kGy for frozen or dry products [11]. Enteric virus inactivation requires a higher dose of gamma irradiation than bacterial pathogens [32,72,77]. The level of irradiation required to eliminate 90% of the hepatitis A virus, poliovirus, and rotavirus in shellfish was $<3.1$ kGy [63], but 6.8 kGy was required to inactivate coxsackievirus type B2 from ground beef [98]. In-shell molluscan shellfish experience high mortalities and reduced shelf-life when subjected to the levels of irradiation necessary to eliminate the enteric viruses. When poliovirus was suspended in water, 90% of the viruses was inactivated with 1.92 kGy, but 3.35 kGy was required to inactivate viruses in wastewater sludges [49]. In many cases, 90% inactivation may not be sufficient to eliminate the threat of illness, since only a few virus particles may be sufficient to elicit illness.

Ionizing radiation has the ability to penetrate foods and kill the microbes within; however, the opposite is true for UV irradiation which only inactivates viruses in direct contact with the light. Germicidal lamps, operating at a wavelength around 254 nm, are effective in reducing viruses on the surfaces of foods. Rotavirus and poliovirus demonstrated similar rates of inactivation from UV; however, these viruses required three to four times the dose of UV necessary for *Escherichia coli* inactivation [21].

Microwave energy inactivates viruses primarily as a function of the heat produced. Poliovirus added to infant formula was eliminated by microwaving [53]; however, the amount of microwave heating required to inactivate enteric viruses will probably vary depending on product composition [15]. Epidemiological evidence that microwaving of foods inactivates hepatitis A virus was reported [71]. A major limitation of microwave processing is that it heats foods unevenly, often leaving cold spots where viruses may survive. Thoroughly stirring or frequently turning foods during microwave processing can reduce the effects of unbalanced heating and ensure greater levels of virus reduction.

Dessication/dehydration
Viruses subjected to drying are generally perceived as more susceptible to inactivation than viruses stored in moist environments. Viruses from raw sewage sludge and wastewater were inactivated more quickly during drying [18,45]. Several enteric viruses were inactivated faster on a variety of porous and non-porous surfaces at low relative humidity [1], although there is one report that the infectivity of hepatitis A virus is inversely proportional to the level of relative humidity [66]. Airborne survival of enteric viruses is enhanced under conditions of high relative humidity [47]. Freeze-drying reduced poliovirus in potato salad, beef and vegetables, beef pot roast, chicken with gravy, and salmon salad by three to four orders of magnitude [40].

Depuration and relaying
Molluscan shellfish may undergo relaying, a process where shellfish are removed from marginally contaminated waters (as determined by coliform levels) and replanted in waters classified by state health authorities as approved (reviewed in Ref. [84]). Once in clean waters, shellfish will purge at least some of their contaminants, provided that the shellfish are physiologically active and the water conditions, temperature, salinity, and dissolved oxygen are favorable. A similar purging process, known as depuration, is conducted commercially in tanks of clean seawater. The water is usually recirculated through a UV or ozone disinfection system which kills or inactivates any bacteria or viruses present in the water (reviewed in Refs. [84,85]). Relaying requires extended periods (often 10 days to 2 weeks or more), whereas depuration may be completed within 48–72 h. In either case, viruses are reduced in shellfish, but are not totally eliminated; both depuration and relaying are more efficient in eliminating potential bacterial pathogens like *E. coli* and *Salmonella* spp. than the enteric viruses. Depurated shellfish can cause enteric virus illness, either as a consequence of improper depuration controls or due to insufficiency in the process itself [5,37]. For these reasons, only lightly contaminated shellfish should be subjected to depuration; more heavily contaminated shellstock should be relayed for extended periods. Shellfish-related industries should...
appreciate the benefits of depuration in reducing bacterial contaminants and intestinal sediment; however, it must be recognized that there are no guarantees that shellfish depurated according to current technologies will be virologically safe.

**Improved handling techniques**

Increased automation of processing lines can significantly reduce contact between product and hands. Robotic technology provides for rapid and efficient processing of foods with minimal opportunity for hand contamination. For operations where hand contact of the food is common, proper disinfection of hands, gloves, or frequent replacement of disposable gloves may be necessary to reduce the opportunity for product contamination. Simple processing techniques to minimize direct human contact with foods can further safeguard the food supply. Perhaps, the simplest and most practical example of this concept is to mix salads, coleslaw, and similar products with a spoon or fork rather than by hand. Another example is to use clean food processors to slice and dice vegetables or cheeses destined for raw consumption rather than cut them by hand. Clean bowls or tubs should be used for mixing rather than sinks which are virtually impossible to thoroughly disinfect. At least one outbreak of foodborne Norwalk-like illness was associated with mixing potato salad in a sink [78].

Self-service, buffet-style restaurants should take precautions to protect foods from contamination by consumers. Too often, serving utensils at salad bars are so small that they fall into the food trays and have to be “fished out” by the consumer, usually using their fingers. Long-handled spoons, forks, and tongs reduce the possibility of direct consumer contact with foods. Single-use containers for salad dressings and condiments eliminate customer handling and possible contamination that may occur in multi-use containers. Sneez guards are commonly used and, in most cases, mandated around buffet tables to prevent accidental contamination of foods by customers or servers.

**Personal hygiene**

Poor personal hygiene remains one of the greatest stumbling blocks in food safety. Providing employee education is a key to improving personal hygiene. Thorough disinfection of hands is difficult, since most chemicals are too harsh for the skin at the concentrations required for virus inactivation [23]. The use of disposable gloves remains a practical and inexpensive alternative to repeated hand disinfection [80]. Automatic or foot-controlled faucets in rest rooms would also reduce the possibility of recontaminating hands after washing. Frequent disinfection of rest room doors and door handles could further retard the spread of viruses. Restrooms should be maintained in a sanitary condition since they have been linked to the spread of enteric virus illness [43]. Although gloves, aprons, and good hygienic practices should reduce the risk of virus transmission to foods, people occasionally forget or disregard safety practices. Signs placed in strategic locations throughout the facility can serve as a reminder of acceptable and unacceptable practices.

**Immunizations**

Vaccines are available for hepatitis A virus, but there is none currently available for the Norwalk-like viruses, astroviruses, or rotaviruses. The RotaShield vaccine (Wyeth–Lederle Vaccines and Pediatrics) for rotavirus was licensed in the United States for oral administration to infants [9,44], but was recently withdrawn from the market due to unforeseen bowel obstructions occurring in children who had received the vaccine. Immunizations of individuals who routinely handle foods could reduce the incidence of foodborne illness; however, conventional wisdom dictates that, rather than target at-risk groups, more broad-based immunization programs designed around a childhood immunization strategy would provide a sustained reduction of hepatitis A within the population. States with a higher-than-average incidence of hepatitis A should consider implementation of immunization programs for children. Two hepatitis A vaccines are available in the United States for children 2 years old and older: the Havrix® (1992 — SmithKline Beecham) and the Vaqta® (1996 — Merck) [6]. Progress is being made on the development of a vaccine against hepatitis E virus [109]; however, its use in the United States is not warranted at this time. If the global distribution of hepatitis E changes, as might be expected with an ever-widening influx of foreign travel and demand for imported foods, hepatitis E immunizations may become necessary. The magnitude of the Norwalk-like outbreaks in the United States demonstrates the need for an efficacious vaccine; however, there have been obstacles to the development of such a vaccine [30].

**Monitoring tools**

The detection of foodborne viruses can be a difficult task. Procedures are available to extract and concentrate viruses from the surfaces of produce and from meats and shellfish [87]; however, most extraction and assay procedures are time-consuming and of limited utility in routine monitoring programs. Traditional assay techniques rely on the propagation of “indicator” viruses since Norwalk-like viruses cannot be propagated in cell cultures, and hepatitis A virus is very difficult to assay. For many years, it was possible to test for the presence of poliovirus in foods since the vaccine strains, commonly shed in the stools of recently immunized children, were indicative of the presence of fecal pollution of human origin and of the possible presence of hepatitis A, Norwalk, and other human enteric viruses for which tests were not available. The efforts of the World Health Assembly have been highly effective in eradicating poliomyelitis from most of the world. The recent elimination of the use of oral polio vaccines in some countries has reduced poliovirus levels in the environment and negated the use of poliovirus as a potential indicator organism.

The advent of molecular biology has led to a plethora of techniques to assay viruses; however, the extraction of viruses from the foods remains a difficult task [87]. Molecular techniques are excellent for epidemiological investigations and are amenable for detecting a wide variety of viruses including hepatitis A and E, the caliciviruses, and astroviruses. Unfortunately, molecular techniques have limited usefulness in predicting the virological safety of foods [86]. Molecular techniques generally involve RT-PCR to detect hepatitis A and Norwalk-like viruses; however, the procedure cannot differentiate between infectious and inactivated viruses, and is unable, under most circumstances, to provide a quantitative measure of the level of virus present [86].
New, innovative approaches are needed to identify the presence of infectious viruses in water and foods. Improved cell culture techniques are needed to propagate wild-type hepatitis A and Norwalk-like viruses. Currently, cell-culture-adapted strains of hepatitis A virus are available for laboratory studies and a feline calicivirus model is available for studies of Norwalk-like viruses. With additional studies on virus–receptor interactions and the use of alternative cell cultures or treatments, it may become possible in the future to directly monitor for hepatitis A and E viruses, caliciviruses, astroviruses, and other viral pathogens in foods.

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