Journal of Food Protection, Vol. 78, No. 8, 2015, Pages 1597–1617
doi:10.4315/0362-028X.JFP-14-487

Review

Thermal Inactivation of Foodborne Enteric Viruses and Their Viral Surrogates in Foods

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MS 14-487: Received 9 October 2014/Accepted 25 February 2015

ABSTRACT

Foodborne viruses, in particular human norovirus and hepatitis A virus, are the most common causes of food-associated infections and foodborne illness outbreaks around the world. Since it is currently not possible to cultivate human noroviruses and the wild-type strain of hepatitis A virus in vitro, the use of a variety of viral surrogates is essential to determine appropriate thermal processing conditions to reduce the risk associated with their contamination of food. Therefore, the objectives of this review are to (i) present pertinent characteristics of enteric foodborne viruses and their viral surrogates, (ii) discuss the viral surrogates currently used in thermal inactivation studies and their significance and value, (iii) summarize available data on thermal inactivation kinetics of enteric viruses, (iv) discuss factors affecting the efficacy of thermal treatment, (v) discuss suggested mechanisms of thermal inactivation, and (vi) provide insights on foodborne enteric viruses and viral surrogates for future studies and industrial applications. The overall goal of this review is to contribute to the development of appropriate thermal processing protocols to ensure safe food for human consumption.

Foodborne enteric viruses are agents of nonbacterial acute gastroenteritis in humans *(14)*. In the United States, it is estimated that 31 pathogens cause 9.4 million foodborne illnesses, 128,000 hospitalizations, and 3,000 deaths annually *(33)*. Viruses alone reportedly cause an estimated 58% (5.5 million) of those foodborne illnesses, 26% of hospitalizations, and 11% of the deaths *(111)*. Viruses that are associated with foodborne and waterborne diseases include human noroviruses, hepatitis A virus (HAV), rotaviruses, hepatitis E virus, adenoviruses, sapoviruses, astroviruses, Aichi virus, parvoviruses, and other enteroviruses *(35)*. Even though gastroenteritis caused by viruses is generally ranked as the primary cause of foodborne illness in the United States, food and environmental samples have not been routinely tested for virus contamination *(64)*.

Viruses have properties that are uniquely different than those of bacterial pathogens and mainly contain either RNA or DNA enclosed in a protein coat or capsid *(54, 92)*. The capsid functions as the primary protective barrier for the viral particle or virion. Most viruses are enclosed by membrane-containing envelopes that are composed of lipid, protein, and glycoprotein. The membranous structure of the envelope can be maintained only in aqueous solutions *(3)* and is readily disrupted by heat, drying, acidic conditions, detergents, and solvents such as ether, which inactivate the virus. As a result, enveloped viruses must remain wet and are generally transmitted in fluids, respiratory droplets, blood, and tissue. Most enveloped viruses cannot survive the harsh conditions of the gastrointestinal tract. Unlike enveloped viruses, the outer coat of nonenveloped viruses is primarily composed of protein, and these viruses can retain their infectivity even when dry. The nonenveloped viruses are very stable to treatments involving temperature, acid, detergent, proteases, and drying *(3)*. Foodborne viruses are typically nonenveloped and often have a low infectious dose; as few as 10 virus particles can produce illness *(86)*. The ability of viruses to persist in the environment and foods coupled with low infectious doses suggests that even a small amount of contamination may pose a significant health risk to the public.

Viruses are transmitted by contaminated food or water, through person-to-person contact, and via cross-contamination from surfaces *(86, 124)*. Foods at risk for the presence of enteric viruses include those primarily subject to handling, such as leafy vegetables, deli items, and other ready-to-eat foods that do not undergo further processing, and those subject to environmental contamination, such as seafood and fresh produce *(109)*. Due to the obligate intracellular parasitic nature of viruses, they cannot multiply in the environment or in foods. Therefore, typical methods used to control bacterial growth in food products and current food hygiene guidelines that rely on prevention of bacterial growth are relatively ineffective against viruses *(57, 77)*.

Thermal processing is one of the main methods utilized in the food industry for preservation of food materials *(113)*. One of the primary goals of thermal processing is to inactivate spoilage and pathogenic microorganisms and produce a safe product with enhanced or extended shelf life *(89)*. The *D*-value is the time at a given temperature necessary to reduce a microbial population present in a defined

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medium by 90% and is indicative of the thermal resistance of a microorganism at a constant temperature. The *z*-value is the temperature increase (or decrease) necessary to reduce (or increase) the *D*-value by 90% and is indicative of the temperature dependence of microbial inactivation *(74)*. Knowledge of the thermal inactivation parameters (*D*- and *z*-values) for a particular microorganism makes it possible to design effective thermal processes *(74, 89)*. While the thermal inactivation kinetics of foodborne bacterial pathogens has been well studied, there is limited information about the thermal inactivation kinetics of foodborne enteric viruses *(99)*. In fact, there is no specific U.S. regulation addressing the minimum time-temperature combination for inactivating viruses in contaminated food. Thus, determination of the thermal inactivation kinetics of foodborne enteric viruses can contribute to improving strategies for the control of virus contamination in foods using thermal processing. The objectives of this review are to (i) present pertinent characteristics of enteric foodborne viruses and viral surrogates, (ii) discuss the viral surrogates currently used in thermal inactivation studies and their significance and value, (iii) summarize available data on the thermal inactivation kinetics of enteric viruses, (iv) discuss factors affecting the efficiency of thermal treatment, (v) discuss suggested mechanisms of thermal inactivation, and (vi) provide insights on foodborne enteric viruses and viral surrogates for future study and industrial applications.

FOODBORNE ENTERIC VIRUSES

Human norovirus. Human noroviruses are the leading cause of outbreaks and sporadic cases of acute gastroenteritis worldwide *(14, 63,* 65*)*. Noroviruses belong to the *Caliciviridae* family, which is composed of five genera: *Norovirus, Sapovirus, Lagovirus, Vesivirus,* and *Nebovirus (39)*. The first two genera contain primarily human viruses, while the other genera contain animal viruses.

Human noroviruses are nonenveloped RNA viruses, approximately 27 to 38 nm in diameter, which to date cannot be cultivated in animal cell culture systems, posing a problem for experimental and food-related research *(64)*. Noroviruses are icosahedral in shape and contain single-stranded positive-sense RNA genomes ranging in size from 7.4 to 8.3 kb. Excluding the 3′ end of the genome, which contains a polyadenine tail, the norovirus genome sequence is 7,642 nucleotides in length *(54)*. This genome contains three open reading frames (ORFs), which encode structural and nonstructural genes *(50)*. ORF1 (nucleotides 146 to 5,359) is the largest ORF, corresponding to ca. 1,700 amino acids, and encodes six nonstructural proteins: p48 (responsible for replication), NTPase (nucleoside triphosphatases), p22 (precursor in the proteolytic processing pathway), VPg (viral protein that binds to the 5′ end to initiate translation), $3CL^{pro}$ (protease), and RdRp (RNA-dependent RNA polymerase) *(37, 50, 54)*. It is speculated that VPg is covalently linked to the viral RNA, caps the 5′ end, and may function in transporting to negative strand synthesis sites *(50)*. ORF2 (nucleotides 5,346 to 6,935) encodes the major viral structural protein (VP1) of approximately 60 kDa that folds into an S (shell) and a P (protruding) domain. The P domain is further divided into P1 and P2. P2 is the most hypervariable region of the genome and is responsible for histoblood group antigen (HBGA) receptor binding *(37, 50, 54)*. ORF3 (nucleotides 6,938 to 7,573) encodes a minor structural protein (VP2) with an unknown function, but in vitro studies have suggested that this gene regulates the expression and stability of VP1 *(10, 50, 54)*.

Based on the molecular characterization of complete capsid gene sequences, noroviruses are classified into five genogroups: GI (prototype is Norwalk virus), GII (prototype is Snow Mountain virus), GIII (prototype is bovine enteric calicivirus), GIV (prototypes are Alphatron and Ft. Lauderdale viruses), and GV (prototype is murine norovirus) *(54, 136)*. Strains of three genogroups, GI, GII, and GIV, are found in humans, and GIII and GV strains are found in cattle and mice, respectively. Among the genogroups infecting humans, GII is predominant and is responsible for approximately 73% of human norovirus illnesses *(136)*. Siebenga et al. *(112)* investigated the sequences of epidemic norovirus strains collected from 1987 to 2008 and estimated the evolutionary and population dynamics of GII.4 noroviruses by using Bayesian coalescent analysis. The observed pattern of continually emerging novel variants of GII.4, causing elevated numbers of infections, is therefore a cause for concern *(112)*. The transmission of human noroviruses occurs by three general routes: person-to-person, via food, and via water. Person-to-person transmission might occur directly through the fecal-oral route, by ingestion of aerosolized vomitus, or by indirect exposure via fomites or contaminated environmental surfaces. Foodborne transmission typically occurs by contamination from infected food handlers during preparation or service but might also occur further upstream in the food distribution system through agricultural contamination by human feces *(52)*. Drinking water may serve as a vehicle of norovirus transmission, and large community outbreaks can occur *(135)*. These outbreaks often involve water that becomes contaminated from septic tank leakage or sewage or from breakdowns in chlorination of municipal systems *(9, 87)*.

It is known that human noroviruses bind to the HBGAs in human intestinal epithelial cells, but the site of replication has not yet been established, although it is assumed to be the small intestine *(75)*. Recently, B cells were identified as a cellular target of noroviruses and enteric bacteria as a stimulatory factor for norovirus infection in the presence of free HBGA or HBGA-expressing bacteria such as *Enterobacter cloacae* and *Escherichia coli (79)*. As of this study, no additional research has been published to demonstrate replication of human noroviruses in cell culture systems in a reproducible manner; thus, the cell tropism of human noroviruses and the development of an in vitro infection model has remained elusive.

Norovirus infection in humans is characterized as a self-limiting gastrointestinal infection with symptoms that include nausea, vomiting, diarrhea, malaise, abdominal pain, muscle pain, anorexia, headache, and low-grade fever. Symptoms generally begin 1 to 2 days following consumption of contaminated foods or water and persist for 1 to 8

days *(64)*. While no antiviral drugs for the prevention or treatment of norovirus infections are approved for human use, human trials for virus-like particle (VLP)–based vaccines show promise in both immune response and protection studies, and vaccines are expected to become available in the next 5 to 10 years *(126)*. Ongoing work including identification of important norovirus capsid antigenic sites, development of improved model systems, and continued studies in humans will allow improvement of future vaccines *(47)*. Detailed descriptions of antiviral approaches for controlling human noroviruses have been published in recent reviews *(80, 82)*.

HAV. HAV is the second most common cause of enteric viral hepatitis, with a high percentage of hospitalizations (32%) and deaths (2%) *(111)*. HAV belongs to the *Picornaviridae* family, which comprises five genera: *Hepatovirus, Enterovirus, Rhinovirus, Cardiovirus,* and *Aphthovirus (110)*.

HAV is a nonenveloped RNA virus ca. 27 to 32 nm in diameter. It is icosahedral in shape and contains a single-stranded positive-sense 7.5-kb RNA genome *(53, 54)*. Unlike human noroviruses, HAV has a genome with only one ORF (ORF1), which is divided into three regions designated P1, P2, and P3. The P1 region encodes for three major viral capsid proteins VP1, VP2, and VP3. The P2 and P3 regions encode for nonstructural proteins required for RNA replication and virion formation *(78, 98)*.

Based on the molecular characterization of capsid gene sequences, HAV may be classified into seven genogroups. Strains of four genogroups, GI, GII, GIII, and GVII, are found in humans, and GIV, GV, and GVI strains are found in simians *(108)*. The most prevalent genotype, GI, and its subgenotypes include 80% of human strains *(85)*. Only one serotype of HAV has been identified to date, and a single exposure can render life-long immunity in an individual *(4)*.

As with other enteric viruses, HAV is transmitted directly from person to person by the fecal-oral route or indirectly through contaminated food, water, or environmental surfaces. Since as many as 10^9 viral particles can be shed in 1 g of stool, direct or indirect contact with feces, emesis, or their aerosolized droplets are important routes of transmission *(36)*. As the infectious dose is very low (10 to 100 virus particles), hands or surfaces that appear clean can still harbor infectious material, contributing to virus spread *(96)*. Contamination of food can occur anywhere along the "farm to fork" continuum that involves human contact or indirectly through fecal contamination of water that comes in contact with foods. Since the capsids of HAV have properties that promote survival for long periods under harsh conditions such as desiccation, freezing, and extremes in pH, these viruses are well adapted to survival in and on foods *(1, 2, 55, 73, 95)*.

After infection, HAV illness spans four phases. The first phase is characterized by viral replication in the body without symptoms and lasts an average of 28 to 30 days *(54, 64)*. The second phase, known as prodromal or preicteric, is characterized by an onset of symptoms including anorexia, vomiting, fatigue, and jaundice and lasts an average of 5 to 7 days *(24)*. The third phase is characterized by the onset of jaundice and an enlarged liver lasting up to 28 days. During the final phase, symptoms resolve and liver enzymes returns to normal.

Currently, there is no specific treatment available for HAV infection. However, symptoms can be alleviated by appropriate patient care. For prevention, immunoglobulin therapy is effective when administered to individuals within 2 weeks of viral exposure (through passive immunity). Inactivated and heat-killed vaccines against HAV also are commercially available and provide >20-year or lifetime immunity against HAV infection *(127)*. The Centers for Disease Control and Prevention *(34)* recommends routine vaccination against HAV for children 12 to 23 months of age. When economically feasible, vaccination of food handlers is recommended to prevent transmission of HAV and to prevent HAV infection outbreaks *(53, 54)*.

Other foodborne enteric viruses. Adenovirus, rotavirus, hepatitis E virus, and Aichi virus have also been implicated in outbreaks of foodborne illnesses but much less frequently than human norovirus or HAV *(90, 134)*. The general characteristics of these enteric viruses are given in Table 1.

THERMAL INACTIVATION OF FOODBORNE ENTERIC VIRUSES

Viral surrogates. Traditionally, surrogate microorganisms are used as nonpathogenic substitutes or replacements for pathogenic microorganisms to validate the efficacy of a food preservation process, often a thermal process. Surrogates should have physiological and resistance characteristics similar to those of the pathogens of interest. Although they should be nonpathogenic for humans, they may be pathogenic for animals *(26)*.

In contrast to the traditional use, surrogates for enteric viruses are used for a very different reason. To date, all attempts to propagate human norovirus and wild-type strains of HAV in routine laboratory cell culture or primary tissue cultures have been unsuccessful *(56, 107)*. Straub et al. *(119, 120)* used a three-dimensional organoid model of a human small intestinal epithelium cell line (Int-407) and an epithelial colon rectal adenocarcinoma cell line (Caco-2) to demonstrate human norovirus replication. However, multiple independent attempts to replicate these cell culture systems have failed, highlighting the complexity and difficulty of developing a reproducible in vitro cell culture system for human norovirus *(67, 101, 123)*. Thus, viral surrogates have played an important role as indicators for inactivation of foodborne enteric viruses to aid in design and validation of food processing systems *(13)*. The ideal surrogate should have a structure and size similar to those of the target, be cultivable in the laboratory, be slightly more resistant to treatments, be nonpathogenic, mimic survival and persistence characteristics, and be transmitted by the fecal-oral route *(26)*. Surrogates for pathogenic foodborne enteric viruses include HAV, feline calicivirus, murine norovirus, bacteriophage MS2, Tulane virus, porcine sapovirus,

TABLE 1. *General characteristics of common foodborne enteric viruses*[a]

Characteristic	NoV	HAV	HEV	Rotavirus	Adenovirus	Aichi virus
Classification						
Baltimore class	Group IV	Group IV	Group IV	Group III	Group I	Group IV
Family	*Caliciviridae*	*Picornaviridae*	*Hepeviridae*	*Reoviridae*	*Adenoviridae*	*Picornaviridae*
Genus	*Norovirus*	*Hepatovirus*	*Hepevirus*	*Enterovirus*	*Adenovirus*	*Kobuvirus*
Capsid						
Envelope	No	No	No	No	No	No
Virion diameter (nm)	27–38	27–32	27–34	30	90–100	27–30
Isoelectric point	5.5–6.0	2.8	3.5	4–4.5, 6.6–7.5	9.7	3.5
Host receptor	HBGA, heparin sulfate, or B cells	TIM-1	HSPGs	PVR (CD55)	CD46, CAR	GM1b
Genome						
Composition[b]	(+) ssRNA	(+) ssRNA	(+) ssRNA	dsRNA	dsRNA	(+) ssRNA
Architecture	Linear	Linear	Linear	Linear	Linear	Linear
Size (kb)	7.5	7.5	7.2	7.5	26–48	8.2
Route of transmission	Fecal-oral	Fecal-oral	Fecal-oral	Fecal-oral	Fecal-oral	Fecal-oral
Incubation time	24–48 h	4 wk	2–9 wk	2–4 days	3–10 days	24–48 h
Duration time	12–72 h	2–6 mo	2–6 mo	3–8 days	7 days	12–72 h
Symptoms	Diarrhea, nausea, vomiting, abdominal pain	Malaise, dark urine, nausea, vomiting, jaundice	Malaise, jaundice, anorexia, vomiting, abdominal pain, fever	Vomiting, diarrhea, dehydration, fever	Vomiting, diarrhea	Diarrhea, abdominal pain, nausea, vomiting
Clinical features	Gastroenteritis	Hepatitis	Hepatitis	Gastroenteritis (children)	Gastroenteritis (children)	Gastroenteritis
Current therapeutics	No specific treatment	No specific treatment	No specific treatment	No specific treatment	No specific treatment	No specific treatment
Vaccine	No	Yes	No	Yes	Yes	No
Detection method[c]	RT-PCR, ELISA, NASBA, RT-LAMP	RT-PCR, ELISA, NASBA, RT-LAMP	RT-PCR, ELISA, NASBA, RT-LAMP	RT-PCR, ELISA, NASBA, RT-LAMP	RT-PCR, ELISA, NASBA, RT-LAMP	RT-PCR, ELISA, NASBA, RT-LAMP

[a] NoV, norovirus; HAV, hepatitis A virus; HEV, hepatitis E virus.
[b] (+), positive sense; ss, single stranded; ds, double stranded.
[c] RT-PCR, reverse transcription PCR; ELISA, enzyme-linked immunosorbent assay; NASBA, nucleic acid sequence–based amplification; RT-LAMP, RT loop-mediated isothermal amplification.

and poliovirus. Characteristic of these viral surrogates are shown in Table 2.

The lack of a suitable cell culture based assay for infectious wild-type strains of HAV has led the research community to focus on appropriate surrogates. There are a few strains of HAV (HM-175, HAS-15, and MBB 11/5) that are cell culture adaptable and maintained using fetal rhesus monkey kidney (FRhK-4) and/or human fetal lung fibroblast (MRC-5) cells. Due to their resistance to environmental stresses such as acid, heat, drying, pressure, disinfectants, and UV radiation, these strains have been used as surrogates in a variety of inactivation studies *(94, 106)*.

Feline calicivirus (FCV) is a respiratory virus and was the first animal virus surrogate used in laboratories to mimic human noroviruses *(51)*. It is a member of the genus *Vesivirus* in the *Caliciviridae* family and is a nonenveloped RNA virus that is approximately 35 to 39 nm in diameter. FCV is icosahedral in shape and contains a single-stranded positive-sense RNA genome of around 7.5 kb in size. Similar to human noroviruses, FCV also has three ORFs. Since it is a respiratory virus and sensitive to low pH (2.0 to 4.0), FCV may not adequately mimic the survival of human noroviruses in the environment or food *(30)*.

Murine norovirus (MNV-1) is also a member of the *Caliciviridae* family and the first virus from the genus *Norovirus* reported to be cultivated in cell culture *(133)*. It has greater genetically similarity to human norovirus than does FCV since it is in the same genus *(71, 76)*. MNV-1 has immunological, biochemical, genetic, and molecular properties that are very similar to those of human noroviruses. MNV-1 is an icosahedral, nonenveloped, single-stranded RNA virus with three ORFs and is 28 to 35 nm in diameter *(133)*. Although it causes a disease in mice that is different from the human disease, it is transmitted via the fecal-oral route (similar to human noroviruses and unlike FCV) and is less sensitive to pH within the range of 2.0 to 10.0 *(30)*. In one of the first studies on thermal inactivation, FCV-F9 and MNV-1 were reported to have similar heat resistance at 63°C *(30)*.

Another potential human enteric virus surrogate is the bacteriophage MS2. A bacteriophage is a virus that infects only bacterial cells, and MS2 infects *E. coli* ATCC 15597B. MS2 is a single-stranded RNA virus with icosahedral symmetry. It belongs to the *Leviviridae* family in group 1 of the RNA coliphages *(29, 46)*. MS2 is commonly found in sewage, is 27 to 34 nm in diameter, and is adapted to the intestinal tract *(46)*. Dawson et al. *(46)* studied the survivability of MS2 on fresh produce and concluded that MS2 is an ideal surrogate for human norovirus and HAV because it can survive for prolonged periods on environmental surfaces. In another study, Black et al. *(13)* investigated six coliphages (T4, MS2, Qβ, λ imm 434, λ cI 857, and λ cI 857A) as pressure surrogates for enteric viruses. Even though MS2 showed some potential as a surrogate for enteric viruses, it did not display enough similarity to the target viruses to be considered an ideal surrogate. Consequently, MS2 may not be a good surrogate for enteric viruses in broader validation studies of high pressure processing. In the current literature, there is no study about the suitability of MS2 as a surrogate for thermal inactivation.

A recently discovered calicivirus with potential for use as a surrogate is Tulane virus (TV). It was isolated from the stools of rhesus macaques (*Macaca mulatta*) and represents a new genus, *Recovirus (60)*. TV has been cultivated successfully in rhesus monkey kidney cells (LLC-MK2) *(131)*. Even though TV does not belong to the genus *Norovirus,* sequence analysis has revealed that it is closely related to the GII noroviruses *(59)*. Similar to human noroviruses, TV also bind to HBGAs *(60)*. This characteristic could make TV structurally more similar than MNV-1 to human noroviruses and potentially a good surrogate for these viruses. However, in terms of thermal resistance, TV is apparently more sensitive to heat than is MNV-1 at temperatures of 50 to 65°C *(71)*.

Sapovirus (SaV) is a member of the genus *Sapovirus* in the *Caliciviridae* family and is a nonenveloped RNA virus of approximately 27 to 35 nm. SaV is also icosahedral in shape and contains a single-stranded positive-sense RNA genome around 7.5 kb in size. Similar to human noroviruses, SaV also is transmitted through the ingestion of fecally contaminated material; however, SaV causes gastroenteritis only in gnotobiotic pigs *(61)*. Wang et al. *(130)* reported that SaV has resistance to chlorine treatment and, similar to human norovirus, is stable at pH 3.0 to 8.0. Thus, cultivable SaV is a good surrogate for studying human norovirus contamination and transmission in leafy greens and evaluating potential disinfectants *(130)*.

Poliovirus has also been used as another potential surrogate for enteric viruses because of its similarity in size, shape, and structure. Poliovirus is a member of the genus *Enterovirus* in the *Picornaviridae* family and is a nonenveloped RNA virus that is approximately 30 nm in diameter. It is icosahedral in shape and contains a single-stranded positive-sense RNA genome, about 7.5 kb in size *(72)*. Even though poliovirus showed some potential as a surrogate for enteric viruses in persistence *(6)* and pressure inactivation *(83, 88)* studies, there is limited information about the suitability of poliovirus as a surrogate for thermal inactivation studies *(121)*. Strazynski et al. *(121)* investigated the thermal tolerance of poliovirus type 1 in milk, where the $D_{72°C}$ was 0.44 min. Based on inactivation data obtained for poliovirus, thermal resistance of poliovirus was less than that of HAV. Thus, cultivable poliovirus should be a good surrogate primarily for studying pressure inactivation and persistence.

VLPs have also been used as surrogates to evaluate virus behavior in inactivation studies. Coexpression of viral capsid proteins in baculovirus expression systems results in the assembly of VLPs that maintain the structural and functional characteristics of the native particles, i.e., they resemble a real virus but are noninfectious *(91)*. VLPs have been used as surrogates for viruses in environmental persistence and chemical inactivation studies *(5, 28, 91)*. It has been reported that VLPs are highly stable over a pH range of 3 to 7 and up to 55°C. However, at temperatures above 55°C they undergo distinct phase transitions arising from secondary, tertiary, and quaternary level protein structural

TABLE 2. *Common viral surrogates used in inactivation studies*[a]

Characteristic	HAV	FCV	MNV	MS2	TV	SaV	Poliovirus
Classification							
Family	*Picornaviridae*	*Caliciviridae*	*Caliciviridae*	*Leviviridae*	*Caliciviridae*	*Caliciviridae*	*Picornaviridae*
Genus	*Hepatovirus*	*Vesivirus*	*Norovirus*	*Levivirus*	*Recovirus*	*Sapovirus*	*Enterovirus*
Capsid							
Envelope	No	No	No	No	No	No	No
Virion diameter (nm)	27–32	35–39	35–39	27–34	36	27–35	30
Isoelectric point	2.8			2.2–3.1, 3.3–3.5, 3.9–4.0	2.8	4–4.5, 6.6–7.5	4–4.5, 6.6–7.5
Host receptor	HAVCR1	JAM-1, sialic acid	Sialic acid, glycoproteins	F-pilus	HBGA	Bile acid needed for replication	PVR(CD55)
Host	Monkey or human	Cat	Mouse	*E. coli*	Monkey	Pig	Human
Genome							
Composition[b]	(+) ss RNA	(+) ssRNA	(+) ssRNA	(+) ssRNA	(+) ssRNA	(+) ssRNA	(+) ssRNA
Architecture	Linear	Linear	Linear	Linear	Linear	Linear	Linear
Size (kb)	7.5	7.5	7.5	3.5	6.7	7.5	7.5
Surrogate for	HAV	NoV	NoV	Enteric viruses[c]	NoV	NoV	NoV

[a] HAV, hepatitis A virus; FCV, feline calicivirus; MNV, murine norovirus; MS2, bacteriophage MS2; TV, Tulane virus; SaV, sapovirus; NoV, norovirus.
[b] (+), positive sense; ss, single stranded.
[c] Human enteric viruses such as NoVs, HAV, enteroviruses, and rotaviruses.

perturbations *(5)*. Hence, VLPs are not suitable as surrogates for thermal inactivation studies *(70)*.

EVALUATION OF THERMAL INACTIVATION DATA FOR FOODBORNE ENTERIC VIRUSES

Thermal inactivation kinetics data for foodborne enteric viruses and their surrogates in cell culture media, seafood, fruits, vegetables, herbs, dairy products, and meat products are shown in Tables 3 through 7 and Figures 1 through 4. The temperature ranges studied were 37 to 100, 50 to 100, 4 to 95, 62.8 to 85, and 50 to 72°C for viruses in cell culture medium; seafood; fruits, vegetables, and herbs; dairy products; and meat products; respectively. The viruses used in these studies belonged to two families (*Picornaviridae* and *Caliciviridae*) and five genera (*Hepatovirus, Vesivirus, Norovirus, Recovirus, Sapovirus,* and *Enterovirus*).

Cell culture media. FCV-F9 was the most commonly used viral surrogate in thermal inactivation studies involving cell culture media (Table 3). The effect of thermal treatment on inactivation of FCV-F9 in cell culture media has been investigated in the temperature range of 37 to 80°C.

In general, the *D*-values for FCV-F9 in cell culture media determined by Bozkurt et al. *(17),* Cannon et al. *(30),* Doultree et al. *(51),* Buckow et al. *(25),* and Duizer et al. *(56)* were lower than those reported by Gibson and Schwab *(62)* (Fig. 1A). The difference between the *D*-values of Gibson and Schwab *(62)* and those of other studies is most likely associated with the heat transfer rate and heating system. As stated by Chung et al. *(38),* the differences in container size can potentially lead to differences in the heat transfer rate and thus differences in the apparent *D*-value.

The reported and/or calculated *z*-values for FCV-F9 in cell culture media were 9.29 to 11.54°C, but there were no significant differences observed between the studies *(17, 18, 30, 51, 56)*. However, there was a significant difference between the thermal inactivation data of Croci et al. *(43)* and those of the other studies. Croci et al. *(43)* evaluated the thermal inactivation behavior of FCV in spiked mollusk tissue by comparing cell culture assay (50% tissue culture infective dose [$TCID_{50}$]/ml) and molecular detection (real-time reverse transcription PCR [rRT-PCR]). In terms of the $TCID_{50}$ assay, Croci et al. *(43)* observed the same amount of inactivation (3.5 log units) at both 60 and 80°C after 3 min of thermal treatment and thus the same *D*-value (1.16 min) for both temperatures (60 and 80°C) (Table 3). Compared with other studies where viral inactivation was related to time and temperature, as it is with other microorganisms, Croci et al. *(43)* found no such relationship. They also determined the *D*-value based on rRT-PCR data, for which they calculated a $D_{60°C}$ of 0.13 min and a $D_{80°C}$ of 0.12 min. While the rRT-PCR assay may be useful for viral nucleic acid destruction, it does not provide information on virus infectivity and might be the reason for differences observed between plaque assays in other studies and results obtained with rRT-PCR.

The second most commonly studied viral surrogate during thermal treatment in cell culture media was MNV-1 (Table 3). The reported *D*-values for MNV-1 for 50 to 80°C were 0.15 to 36.28 min. The first thermal inactivation data generated for MNV-1 were reported by Cannon et al. *(30),* who studied survival at 56, 63, and 72°C using the capillary tube method (50 μl) and found *D*-values of 3.47, 0.44, and 0.17 min, respectively. The *D*-values reported by other researchers *(17, 18)* were consistent with those of Cannon et al. *(30)* (Fig. 1B). Bozkurt et al. *(18)* also evaluated the contribution of sample volume (2 ml) to the thermal inactivation behavior of MNV-1. Their reported *D*-values were higher than those obtained in the capillary tube study at 60, 65, and 72°C ($P < 0.05$), but there were no significant differences at 50 and 56°C ($P > 0.05$). The difference in these results may be explained by different heat transfer rates *(17, 18)*. In the capillary tube method, the temperature reaches the desired level almost instantly, while in the 2-ml vial there is a short come-up time to achieve the desired temperature. Even though Hirneisen and Kniel *(71)* also investigated the thermal inactivation behavior of MNV-1 at the same temperatures (50, 55, 60, and 65°C) for 2 min, their reported *D*-values were lower than those of Cannon et al. *(30)* and Bozkurt et al. *(17, 18)* (Table 3). Differences between the result of these studies again might be related to their methods. In their studies, Cannon et al.*(30)* and Bozkurt et al. *(17, 18)* used selected time intervals to determine thermal inactivation data for MNV-1. However, Hirneisen and Kniel *(71)* performed heat treatments at selected temperatures for only 2 min and did not consider any other time intervals. The reported and/or calculated *z*-values for MNV-1 for the studies with consistent *D*-values *(17, 18, 30)* were 9.31 to 12.23°C, and there were no significant differences between the results obtained in these studies (Fig. 1B).

Another commonly reported surrogate in thermal inactivation studies was HAV, which was used in 21% of the studies. The reported and/or calculated *D*-values for 50 to 72°C ranged from 0.88 to 385 min for HAV (Table 3). Similar to FCV-F9 and MNV-1, the highest values were reported by Gibson and Schwab *(62)* (Fig. 1C). As discussed above, the use of larger heating vessels (15 ml) is a likely reason for the observed differences. Most of studies on the thermal resistance of HAV in cell culture media have included only one or two temperatures *(31, 40, 69),* making calculation of *z*-values unreliable. In one of those studies, however, Cappellozza et al. *(31)* reported *D*-values at 60 and 70°C of 2.19 and 1.09 min, respectively, which were consistent with the 2.67 and 1.27 min at the same temperatures reported by Bozkurt et al. *(18)* (Fig 1C). Since Bozkurt et al. *(18)* covered a wide temperature range (50 to 72°C), precise thermal resistance kinetics could be established for HAV. The calculated *z*-values for HAV were 9.99°C by Gibson and Schwab *(62)* based on three temperatures and 12.51°C by Bozkurt et al. *(18)* based on five temperatures. Other surrogates used in inactivation studies included TV and SaV (Table 3). For TV, the calculated *D*-values (50 to 72°C) ranged from 0.65 to 1.8 min *(71, 125)*. Based on their data, the calculated *z*-value for TV ranged from 55.4 to 40°C. The only reported *D*-value for SaV at 56°C was 12.60 min *(130)*.

In direct comparisons between foodborne enteric viruses and viral surrogates in cell culture media, FCV, MNV, TV, and SaV show similar thermal inactivation

TABLE 3. *Thermal inactivation of foodborne enteric viruses and their viral surrogates in cell culture media*

Virus	Enumeration unit	Vol	Temp (°C)	*D*-value (min)	R^2	*z*-value (°C)	R^2	Reference
Feline calicivirus (FCV-F9)	$TCID_{50}$	100 µl	56	8				*51*
			70	0.49	0.90			
			100	0.13				
	$TCID_{50}$	250 µl	37	480		9.87	0.98	*56*
			56	2.7				
			71.3	0.17				
	PFU/ml	50 µl	56	6.40		9.46	0.92	*30*
			63	0.41				
			72	0.12				
	PFU/ml	100 µl	70	1.5				*25*
	$TCID_{50}$	15 ml	37	599		14.01	0.98	*62*
			50	50.6				
			60	14.1				
	RT-PCR	400 µl	60	0.13				*43*
			80	0.12				
	$TCID_{50}$	2 ml	60	1.16				*43*
			80	1.16				
	$TCID_{50}$	100 µl	56	6.09				*130*
	PFU/ml	50 µl	50	20.23	0.98	9.29	0.93	*17*
			56	6.36	0.93			
			60	0.56	0.93			
			65	0.32	0.98			
			72	0.11	0.98			
	PFU/ml	2 ml	50	19.95	0.98	10.97	0.94	*18*
			56	6.37	0.93			
			60	0.94	0.95			
			65	0.72	0.97			
			72	0.21	0.98			
Murine norovirus (MNV-1)	PFU/ml	50 µl	56	3.47		12.23	0.93	*30*
			63	0.44				
			72	0.17				
	PFU/ml	400 µl	80	0.38				*8*
	PFU/ml	100 µl	63	0.9				*69*
			72	<0.3				
	$TCID_{50}$	100 µl	56	12.39				*130*
	PFU/ml	50 µl	50	34.49	0.91	9.31	0.90	*17*
			56	3.65	0.96			
			60	0.57	0.90			
			65	0.3	0.99			
			72	0.15	0.99			
	PFU/ml	2 ml	50	36.28	0.91	10.37	0.92	*18*
			56	3.74	0.92			
			60	1.09	0.94			
			65	0.77	0.96			
			72	0.25	0.97			
	PFU/ml	200 µl	50	2.47		22.83	0.93	*71*
			55	1.18				
			60	0.64				
			65	0.56				
	$TCID_{50}$	2 ml	60	7.79		27.85	0.99	*103*
			85	1.11				
			100	0.28				
Hepatitis A virus (HAV)	$TCID_{50}$	4 ml	60	6.5				*40*
	PFU/ml	100 µl	63	0.6				*69*
			72	<0.3				
	PFU/ml	15 ml	50	385		9.99	0.97	*62*
			60	74.6				
			70	3.84				
	$TCID_{50}$	50 µl	60	2.19				*31*
			70	1.09				

TABLE 3. *Continued*

Virus	Enumeration unit	Vol	Temp (°C)	*D*-value (min)	R^2	*z*-value (°C)	R^2	Reference
	$TCID_{50}$	2 ml	60	6.33		29.67	0.99	*102*
			85	0.98				
			100	0.28				
	PFU/ml	2 ml	50	56.22	0.90	12.51	0.91	*18*
			56	8.40	0.93			
			60	2.67	0.95			
			65	1.73	0.95			
			72	0.88	0.96			
Tulane virus (TV)	PFU/ml	200 μl	50	1.12		55.4	0.86	*71*
			55	1.09				
			60	0.69				
			65	0.65				
	$TCID_{50}$	150 μl	56	11.8		40	0.35	*125*
			63	2.6				
			72	4.3				
Sapovirus (SaV)	$TCID_{50}$	100 μl	56	12.6				*130*
Human norovirus GII (HuNoV GII)	RT-PCR	25 μl	60	25				*43*
			80	5.17				
	ISC-qRT-PCR[a]	300 μl	56	100		20.61	0.90	*129*
			63	25				
			72	3.33				
			100	0.57				

[a] ISC-qRT-PCR, in situ capture quantitative reverse transcription PCR.

characteristics (50 to 100°C). However, HAV was significantly more resistant to thermal treatments at all temperatures (50 to 100°C). Human noroviruses show a higher apparent degree of thermal stability than all cultivable surrogates, including HAV (Table 3). In a novel study in which inactivation conditions for human norovirus were measured by an in situ capture quantitative RT-PCR (ISC-qRT-PCR) assay using HBGAs as viral receptors or coreceptors, human noroviruses (GII.4) had a *D*-value of 0.57 min at 100°C *(129)*. Based on the data presented by Wang and Tian *(129),* the activation energy for human noroviruses was 113 kJ/mole of virion, which is consistent with the required activation energy for disrupting the RNA (96 to 121 kJ/mole) *(15)*. This activation energy value for human noroviruses was relatively lower than that of the cultivable surrogates (171 to 278 kJ/mole of virion) *(18),* bacterial spores (250 to 335 kJ/mole), and vegetative cells (210 to 625 kJ/mole) *(66)*. Since activation energy is a measure of the temperature sensitivity of the microorganism under various conditions, a large activation energy means that small increases in temperature have significantly greater effects on thermal death. Thus, it would prudent to base thermal process design for foods on human noroviruses when there is a potential for contamination by the virus. Even though the ISC-qRT-PCR assay using HBGAs as viral receptors or coreceptors *(129)* is an improved method for human norovirus detection, the site of replication remains elusive, and the method currently is not reliable for investigating host-receptor interaction or virus infectivity. This method awaits confirmation in subsequent studies, and there is still a definite need for comparative research using surrogates alongside human noroviruses and correlating data from qPCR assays of human norovirus with infectivity of norovirus surrogates.

Seafood. Seafood, primarily shellfish, has been the heating medium for 25% of the thermal inactivation studies on enteric viruses (Table 4). This might be expected since many of the reported foodborne illness outbreaks caused by viruses are associated with seafood. Since human norovirus cannot be cultivated in vitro, thermal inactivation studies with these viruses are rare, but they was used in two studies where survival was determined by RT-PCR assays *(43, 68)*.

There is limited information about the thermal inactivation behavior of FCV-F9 in seafood *(22, 43, 115)* (Fig. 2A). The only seafood items used in inactivation studies with FCV were cockles *(115)* and mussels *(22, 115)*. Slomka and Appleton *(115)* investigated the inactivation of FCV-F9 in cockles at 100°C and calculated a $D_{100°C}$ of 0.26 min (Fig. 2A). Croci et al. *(43)* also determined the $D_{60°C}$- and $D_{80°C}$-values of FCV-F9 in mussels as 6.82 and 1.36 min, respectively. Bozkurt et al. *(22)* determined the thermal inactivation kinetics of FCV-F9 in blue mussel homogenate. The calculated *D*-values (50 to 72°C) ranged from 0.07 to 5.20 min, and the reported *z*-value was 11.39°C *(22)* (Fig. 2A). This value was consistent with their previous findings in which the reported *z*-values were 9.29°C in cell culture media *(17),* 9.89°C in spinach *(20),* and 10.91°C in turkey deli meat *(21)*.

For MNV-1, the only foods used in inactivation studies were clams, blue mussel homogenate, and dried mussels *(22, 103, 117)* (Fig. 2B). According to Sow et al. *(117),* the calculated $D_{90°C}$ of MNV-1 in clam was 0.55 min. In contrast, Bozkurt et al. *(20)* reported *D*-values for MNV-1

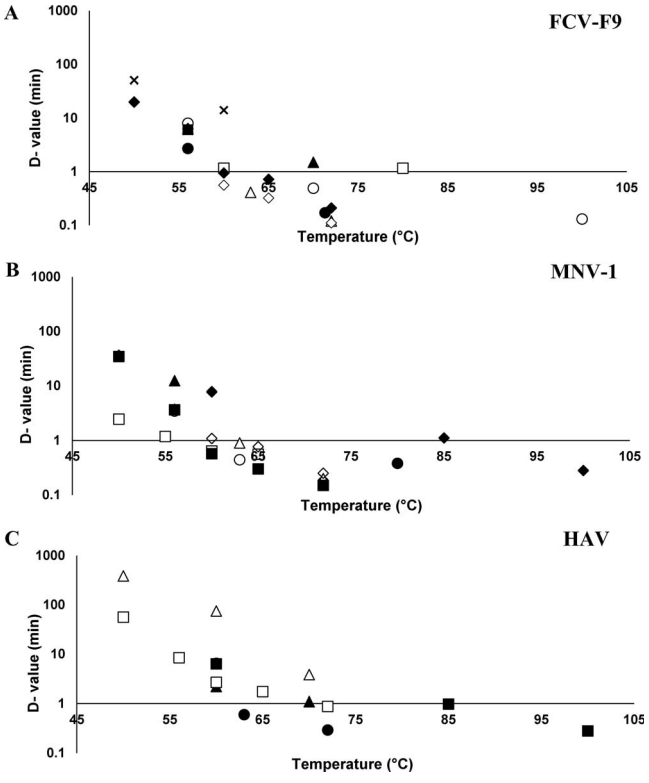

FIGURE 1. *Thermal inactivation of foodborne enteric viruses and their viral surrogates in cell culture medium. (A) Feline calicivirus (FCV-F9): ○, Doultree et al. (51); ●, Duizer et al. (57); △, Cannon et al. (30); ▲, Buckow et al. (25); ×, Gibson and Schwab (62); □, Croci et al. (43); ■, Wang et al. (130); ◇, Bozkurt et al. (17); ◆, Bozkurt et al. (18). (B) Murine norovirus (MNV-1): ○, Cannon et al. (30); ●, Baert et al. (8); △, Hewitt et al. (69); ▲, Wang et al. (130); □, Hirneisen and Kniel (71); ■, Bozkurt et al. (17); ◇, Bozkurt et al. (18); ◆, Park et al. (103). (C) Hepatitis A virus (HAV): ○, Croci et al. (40); ●, Hewitt et al. (69); △, Gibson and Schwab (62); ▲, Cappellozza et al. (31); □, Bozkurt et al. (18); ■, Park and Ha (102).*

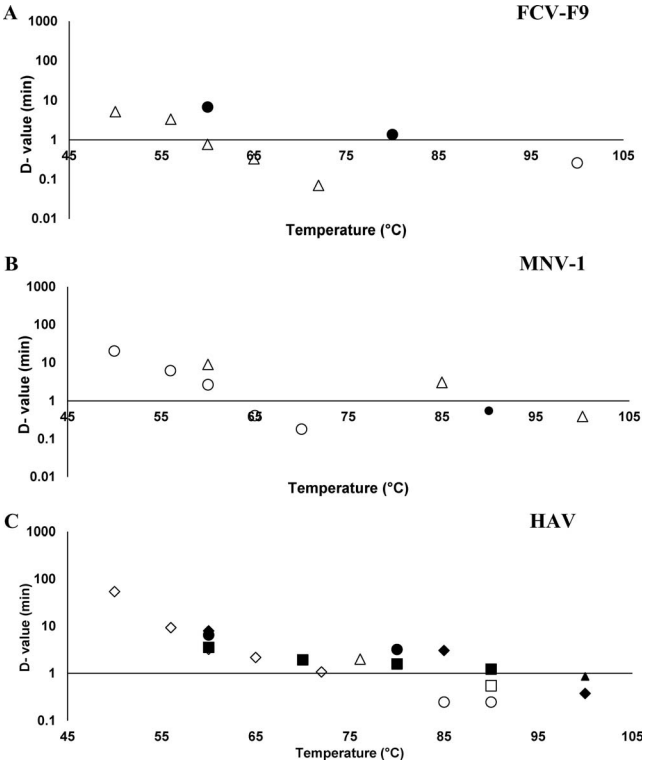

FIGURE 2. *Thermal inactivation of foodborne enteric viruses and their viral surrogates in seafood. (A) Feline calicivirus (FCV-F9): ○, Slomka and Appleton (115); ●, Croci et al. (43); △, Bozkurt et al. (22). (B) Murine norovirus (MNV-1): ○, Bozkurt et al. (22); ●, Sow et al. (117); △, Park et al. (103). (C) Hepatitis A virus (HAV): ○, Millard et al. (97); ●, Croci et al. (40); △, Croci et al. (41); ▲, Hewitt and Greening (68); □, Sow et al. (117); ■, Cappellozza et al. (31); ◇, Bozkurt et al. (19); ◆, Park and Ha (102).*

at 50 to 72°C of 0.18 to 20.19 min, with a *z*-value of 11.62°C. The latter finding was in agreement with the earlier work of Bozkurt et al. *(17, 18, 20)* with similar *z*-values (9.31, 10.37, and 10.98°C).

Thermal inactivation studies for HAV have involved mussels (dried and fresh), clams, and cockles. The most common shellfish used were mussels *(19, 40, 41, 68, 102)*. Croci et al. *(40)* reported that immersion of blue mussels (*Mytilus galloprovincialis*) at 100°C for 2 min was sufficient to achieve complete inactivation (5.6 log units) of HAV. In a subsequent study, Croci et al. *(41)* investigated the resistance of HAV in blue mussels subjected to different domestic cooking methods (mussels hors d'oeuvre, mussels au gratin, and mussels in tomato sauce). They reported a 4-log reduction only in the mussels in tomato sauce, which were boiled for a total of 23 min (100°C) *(41)*. Hewitt and Greening *(68)* stated that treatments at 90°C for 3 min (both steaming and immersion) were sufficient to cause a 3.5-log reduction of HAV in New Zealand greenshell mussels (*Perna canaliculus*). Similarly, Sow et al. *(117)* concluded that application of 90°C for 3 min was sufficient to obtain a 5.5-log reduction of HAV in soft shell clams (*Mya arenaria*). While valuable empirical data were generated in these studies, no thermal inactivation kinetics were established. In a recent study, Bozkurt et al. *(19)* investigated the thermal inactivation kinetics of HAV in blue mussel homogenate (50 to 72°C) and reported *D*-values of 54.17, 9.32, 3.25, 2.16, and 1.07 min at 50, 56, 60, 65, and 72°C, respectively (Fig. 2C).

The reported and calculated *z*-values for HAV was 12.97°C in mussels *(19)* and 68°C in clams *(31)*. According to the study of Cappellozza et al. *(31),* the *z*-values of HAV in cell culture media heated in a thermocycler and in clams heated in an industrial gas oven were completely different at 33 and 68°C, respectively. As the *z*-value is not a function of the heating environment but rather is a characteristic of the microorganism, it should not be greatly different in different heating environments. Thus, the reason for the large differences in *z*-values in this study are unknown but may relate to inactivation under wet and dry conditions. The *z*-value for HAV in mussels as determined by Bozkurt et al. *(19)* was 12.97°C and was consistent with previous findings of 12.51°C in cell culture media *(18)*. The calculated activation energy for HAV in blue mussels was 165 kJ/mole of virion *(19)*. From this study, it was determined that the process time necessary to achieve a 6-log reduction of HAV in mussels in boiling water (100°C) was 2.7 min.

TABLE 4. *Thermal inactivation of foodborne enteric viruses and their viral surrogates in seafood samples*

Virus	Enumeration unit	Sample	Temp (°C)	*D*-value (min)	R^2	*z*-value (°C)	R^2	Reference
Feline calicivirus (FCV-F9)	RT-PCR	Cockles	100	0.26				*115*
	$TCID_{50}$	Mussels	60	6.82				*43*
			80	1.36				
	PFU/ml	Mussels	50	5.20	0.90	11.39	0.97	*22*
			56	3.33	0.92			
			60	0.77	0.95			
			65	0.33	0.97			
			72	0.07	0.99			
Murine norovirus (MNV-1)	PFU/ml	Clams	90	0.55				*117*
	PFU/ml	Mussels	50	20.19	0.95	11.62	0.97	*22*
			56	6.12	0.91			
			60	2.64	0.97			
			65	0.41	0.93			
			72	0.18	0.99			
	$TCID_{50}$	Mussels	60	9.01		29.65	0.90	*102*
			85	3.06				
			100	0.39				
Hepatitis A virus (HAV)	$TCID_{50}$	Cockles	85	0.25				*97*
			90	0.25				
	$TCID_{50}$	Mussels	60	6.5				*40*
			80	3.2				
	$TCID_{50}$	Mussels	76.1	2				*41*
	RT-PCR	Mussels	100	1.58				*68*
	$TCID_{50}$		100	0.86				
	PFU/ml	Clams	90	0.55				*117*
	$TCID_{50}$	Clams	60	3.56		68	0.90	*31*
			70	1.93				
			80	1.58				
			90	1.23				
	PFU/ml	Mussels	50	54.17	0.89	12.97	0.92	*19*
			56	9.32	0.91			
			60	3.25	0.90			
			65	2.16	0.86			
			72	1.07	0.91			
	$TCID_{50}$	Dried mussels	60	7.93		31.94	0.88	*102*
			85	3.05				
			100	0.38				
Human norovirus GII (HuNoV GII)	RT-PCR	Mussels	100	1.3				*68*
	RT-PCR	Mussels	60	25				*43*
			80	4.84				

As stated above, there are very limited thermal inactivation data on actual human noroviruses. Mussels are the only food sample currently reported in the literature to be used in thermal inactivation studies of human noroviruses *(43, 68)*. The reported *D*-values for human norovirus (GII.3) at 60 and 80°C were 25 and 4.84 min, respectively, and the two-point *z*-value based on these data was 28°C *(68)*. The calculated *D*-value for human norovirus (GII.4) at 100°C was 0.93 min *(43),* which was consistent with the 1.3 min calculated from the study of Hewitt and Greening *(68)*.

In general, the thermal inactivation of viruses in seafood samples was considerably lower than that in cell culture media at 100°C (Tables 3 and 4). The efficacy of heat against enteric viruses and viral surrogates in different foods would be expected to vary since food components are known to influence the thermal inactivation of viruses. For seafood, the high protein content may exert a protective effect on enteric viruses against heat *(48)*. Among viral surrogates, both FCV-F9 and MNV-1 had similar thermal inactivation behavior in mussels with $D_{72°C}$ of less than 30 s using a PFU assay (Table 4). $TCID_{50}$ assays indicated slightly higher *D*-values. However, HAV was significantly more resistant to thermal treatment in mussels and clams than were human norovirus surrogates, where *D*-values were ≥10 times higher. The application of a thermal treatment at 100°C for 2 min would result in an approximately 6-log inactivation of HAV as determined by the PFU assay, which is equivalent to a 2-log reduction of human norovirus based on the RT-PCR assay (Table 4). Since inactivation of these viruses in shellfish is dependent upon both their inactivation kinetics and the rate of heat penetration into the shellfish, the determination of adequate industrial thermal processes would require heat penetration experiments.

TABLE 5. *Thermal inactivation of foodborne enteric viruses and their viral surrogates in fruits, vegetables, and herbs*

Virus	Enumeration unit	Sample	Temp (°C)	*D*-value (min)	R^2	*z*-value (°C)	R^2	Reference
Feline calicivirus (FCV-F9)	PFU/ml	Spinach	50	17.39	0.92	9.89	0.93	*20*
			56	5.83	0.93			
			60	0.78	0.89			
			65	0.27	0.91			
			72	0.15	0.98			
	$TCID_{50}$	Basil	75	0.63				*27*
			95	<0.63				
		Chives	75	<0.63				
			95	0.85				
		Mint	75	<0.63				
			95	0.78				
		Parsley	75	0.68				
			95	<0.63				
	RT-PCR	Basil	75	1.53				
			95	1.42				
		Chives	75	1.24				
			95	0.69				
		Mint	75	1.98				
			95	0.47				
		Parsley	75	0.80				
			95	1.78				
Murine norovirus (MNV-1)	PFU/ml	Spinach	80	0.74				*7*
	PFU/ml	Raspberry puree (9.2° Brix)	65	0.44				*8*
			75	0.17				
	PFU/ml	Spinach	50	14.57	0.88	10.98	0.97	*20*
			56	3.29	0.81			
			60	0.98	0.91			
			65	0.4	0.97			
			72	0.16	0.98			
Hepatitis A virus (HAV)	PFU/ml	Strawberry mashes (28° Brix)	85	0.96				*48*
		Strawberry mashes (52° Brix)	80	4.98				
			85	8.94				
	$TCID_{50}$	Basil	75	1.34				*27*
			95	<0.83				
		Chives	75	<0.83				
			95	<0.83				
		Mint	75	1.46				
			95	<0.83				
		Parsley	75	1.21				
			95	1.03				
	RT-PCR	Basil	75	1.62				
			95	0.78				
		Chives	75	1.98				
			95	<0.62				
		Mint	75	1.34				
			95	<0.62				
		Parsley	75	1.19				
			95	0.75				
	PFU/ml	Spinach	50	34.4	0.97	13.92	0.98	*23*
			56	8.43	0.97			
			60	4.55	0.98			
			65	2.30	0.94			
			72	0.91	0.96			
Human norovirus GI (HuNoV GI)	RT-PCR	Basil	75	5.20				*27*
			95	4.90				
		Chives	75	<0.83				
			95	<0.83				
		Mint	75	2.57				
			95	<0.83				

TABLE 5. *Continued*

Virus	Enumeration unit	Sample	Temp (°C)	*D*-value (min)	R^2	*z*-value (°C)	R^2	Reference
		Parsley	75	1.56				
			95	1.58				
Human norovirus GII (HuNoV GII)	RT-PCR	Basil	75	1.71				*27*
			95	1.55				
		Chives	75	1.85				
			95	1.08				
		Mint	75	1.58				
			95	<0.83				
		Parsley	75	1.64				
			95	0.89				

Fruits, vegetables, and herbs. Fruits and vegetables used to determine the thermal inactivation of enteric viruses have included spinach, fruits (raspberry puree and strawberries), and herbs (basil, chives, mint, and parsley) (Table 5). All of these products either have been associated with viral gastroenteritis outbreaks or have the potential to be contaminated by enteric viruses through handling or water. As with seafood, the use of actual human norovirus in studies involving thermal inactivation on produce remains very limited.

Thermal inactivation of FCV-F9 in basil, chives, mint, and parsley was investigated at 75 and 95°C by viral cultural infectivity assays ($TCID_{50}$) and qRT-PCR, and the *D*-values based on the reported thermal data ranged from <0.63 to 0.85 min *(27)* (Fig. 3A). At each temperature, the infectious FCV level determined by virus culture ($TCID_{50}$) was lower than the number of detected infectious RNA copies by RT-PCR assay. Since the RNA extraction procedure did not involve a preliminary step to distinguish infectious and noninfectious RNA particles, the presence of false-negative or false-positive results could explain differences in reported results of the two methods. In addition, only for parsley was the *D*-value determined by RT-PCR assay at 95°C (1.78 min) higher than that at 75°C (0.80 min). The deviation in thermal data could be explained by possible contamination or extraction inefficiency.

For FCV-F9, the other food sample used in thermal inactivation studies was spinach *(20)* (Fig. 3A). At 50, 56, 60, 65, and 72°C, the reported *D*-values ranged from 0.15 to 17.39 min *(20)*. The reported *z*-value was 9.89°C *(19),* and this value was consistent with previous findings in which the reported *z*-values were 9.29°C in cell culture media *(17)* and 11.39°C in blue mussel homogenate *(19)*.

For MNV-1, the only foods that were used in inactivation studies were spinach and raspberry puree (9.2° Brix) *(7, 8, 20)* (Fig. 3B). Baert et al. *(8)* investigated the effect of blanching at a constant temperature (80°C) on the survival of MNV-1. They reported that blanching spinach (80°C for 1 min) resulted in at least a 2.44-log reduction of infectious MNV-1 (Table 5). Even though valuable empirical data were generated in this study, no thermal inactivation kinetics could be established. The only study that reported detailed thermal inactivation kinetics of MNV-1 in spinach was that of Bozkurt et al. *(20)*. The *D*-values for MNV-1 were generally higher than those for FCV in spinach at the same temperatures. The *z*-value of 10.98°C was in agreement with those of Bozkurt et al. *(17–19),* who reported similar *z*-values (9.31 to 11.62°C).

The thermal inactivation kinetics of HAV has been determined in strawberry, basil, chives, mint, parsley, and spinach (Table 5 and Fig. 3C). The reported *D*-values for HAV in strawberry mashes of 28 and 52° Brix at 85°C were 0.96 and 8.94 min, respectively *(48)*. Results indicated that increasing the Brix had a protective effect on thermal inactivation of HAV. Based on the data for strawberry mash of 52° Brix, a two-point *z*-value of 19.67°C could be calculated. Butot et al. *(27)* investigated thermal inactivation of HAV in basil, chives, mint, and parsley at 75 and 95°C and found that HAV was generally more heat resistant than FCV under the same conditions. Unlike FCV, the level of infectious HAV determined by virus culture ($TCID_{50}$) was higher than the number of infectious RNA copies detected by RT-PCR assay for chives, mint, and parsley at each temperature. Since there was no internal control for the RT-PCR assay (or differentiation between infectious and noninfectious viral particles), it is difficult to interpret the difference between the results of the $TCID_{50}$ and RT-PCR assays.

Thermal inactivation of HAV (50 to 72°C) in spinach was investigated by Bozkurt et al. *(23)*. Their reported *D*-values ranged from 34.4 to 0.91 min at 50 to 72°C, and the *z*-value was 13.92°C. These findings were consistent with those of their other studies *(18, 19),* with similar reported *z*-values (12.51 to 12.97°C). The effect of an industrial spinach blanching process on survival of HAV was estimated using the thermal data in the study. According to Singh *(114),* industrial blanching conditions for spinach include the use of steam as a heating medium for 120 to 180 s. Using this information as a basis, blanching of spinach in water at 100°C for 120 to 180 s under atmospheric conditions will result in a >6-log reduction of HAV. It is important to note that steam and hot water (100°C) have different heating characteristics, and the recommendation for use of steam must be validated before actual application of the process, as reported previously *(23)*.

In general, thermal resistance of foodborne enteric viruses and viral surrogates in fruits, vegetables, and herbs was lower than in either cell culture media or seafood. As stated above, the composition of the heating medium influences the sensitivity to thermal inactivation. The presence of more protein in cell culture media and seafood may

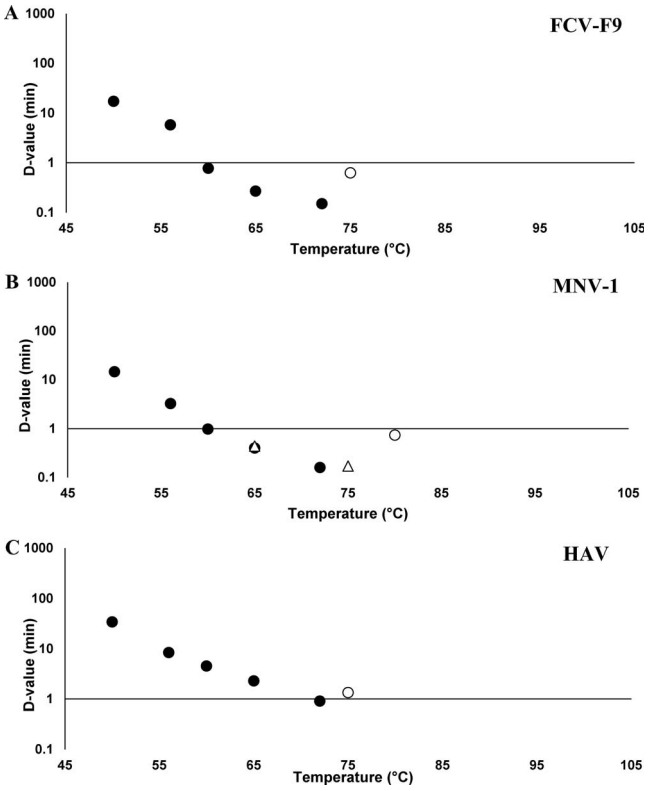

FIGURE 3. *Thermal inactivation of foodborne enteric viruses and their viral surrogates in fruits, vegetables, and herbs. (A) Feline calicivirus (FCV-F9): ○, Butot et al. (27); ●, Bozkurt et al. (20). (B) Murine norovirus (MNV-1): ○, Baert et al. (8); ●, Bozkurt et al. (20); △, Baert et al. (7). (C) Hepatitis A virus (HAV): ○, Butot et al. (27); ●, Bozkurt et al. (23).*

protect enteric viruses from the action of heat, whereas the higher water activity in vegetables and herbs may increase their sensitivity to thermal treatment *(11)*. With fruits, a lower pH or lower water activity may influence inactivation. In strawberry mash with a high sugar content, the heat resistance of HAV was higher than that in spinach, which may indicate protection by lower water activity.

Thermal resistance of human noroviruses GI and GII.4 in basil, chives, mint, and parsley was also investigated at 75 and 95°C by qRT-PCR assay *(27)*. The resistance of human norovirus GI ($D_{75°C}$ = 5.20 min, $D_{95°C}$ = 4.90 min) to thermal inactivation was significantly higher than that of human norovirus GII ($D_{75°C}$ = 1.71 min, $D_{95°C}$ = 1.55 min) on basil at each temperature (Table 5). The same behavior was also observed for mint at 75°C, where the *D*-values of GI and GII.4 were 2.57 and 1.58 min, respectively. Reduction of the human norovirus GI and GII titers after blanching (at 95°C for 2.5 min) was dependent on the type of herb. The greatest reduction in human norovirus GI titers ($D_{95°C}$ < 0.83 min) and GII ($D_{95°C}$ < 0.83 min) was observed for mint, for which the virus titer was reduced by more than 3 log units (Table 5). The same level of reduction (3 log units) of norovirus GI on chives was also observed at 75 and 95°C (<0.83 min) (Table 5) *(27)*. Based on the available thermal data for human noroviruses (GI and GII.4) in the studied food samples (basil, chives, mint, and parsley), it could be concluded that the thermal resistance of human norovirus GI and GII.4 is higher than that of the viral surrogates MNV, FCV, and HAV. While thermal resistance of cultivable surrogates was lower than that of human norovirus, they still have potential value in laboratory validation studies with foods as long as the relative differences are known.

Dairy products. The only dairy product in which foodborne enteric viruses have been tested for their thermal resistance was milk (Table 6). Surrogates used in these studies included HAV, MNV, and poliovirus (Fig. 4A and 4B). For milk at 63°C, the normal temperature for vat pasteurization, the *D*-values ranged from 1 to 10 min *(69, 93, 104)*. At 72°C, the minimum temperature for high temperature–short time pasteurization, the *D*-values for HAV in milk were 7.8 s and <18 s *(69, 104)*. Due to the survival curves at different temperatures, it was not possible to calculate a *z*-value. Bidawid et al. *(12)* investigated the effect of fat concentration (1, 3.5, and 18%) of milk on the thermal resistance of HAV at 71°C (Table 6). These authors concluded that higher fat concentrations provided a protective effect against thermal inactivation of HAV. There was only one study that dealt with thermal inactivation of MNV-1 in milk, and *D*-values of 0.7 and 0.5 min at 63 and 72°C, respectively, were determined (Fig. 4A) *(69)*. Additionally, one study was done on the thermal inactivation of poliovirus in milk, where the $D_{72°C}$ was 0.44 min *(121)*. With the exception of the study by Hewitt et al. *(69),* the current standard of pasteurizing milk at 63°C for 30 min or 72°C for 15 s are not adequate

TABLE 6. *Thermal inactivation of foodborne enteric viruses and their viral surrogates in dairy products*

Virus	Enumeration unit	Sample	Temp (°C)	*D*-value (min)	Reference
Murine norovirus (MNV-1)	RT-PCR	Milk	63	0.7	*69*
			72	0.5	
Hepatitis A virus (HAV)	$TCID_{50}$	Milk	62.8	10	*104*
			71.6	0.13	
	PFU/ml	Milk	85	0.01	*12*
		1% fat milk	71	1.64	
		3.5% fat milk	71	2.08	
		18% fat milk/cream	71	3.16	
	PFU/ml	Milk	63	10	*93*
	$TCID_{50}$	Milk	63	1.1	*69*
			72	<0.3	
Poliovirus (PV)	PFU/ml	Milk	72	0.44	*121*

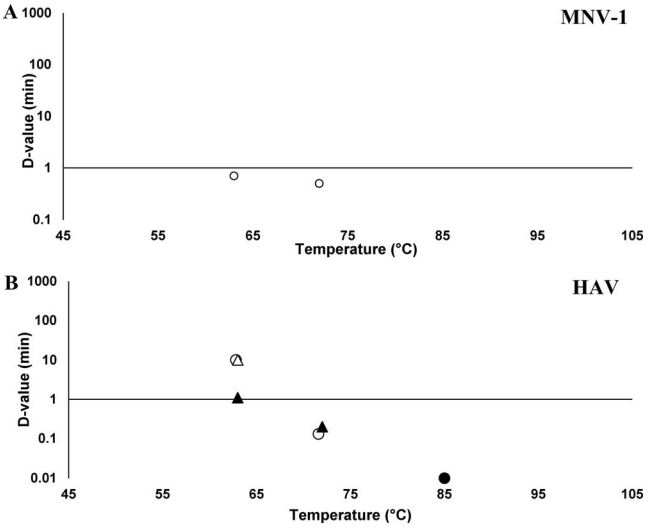

FIGURE 4. *Thermal inactivation of foodborne enteric viruses and their viral surrogates in dairy products. (A) Murine norovirus (MNV-1): ○, Hewitt et al. (69). (B) Hepatitis A virus (HAV): ○, Parry and Mortimer (104); ●, Bidawid et al. (12); △, Mariam and Cliver (93); ▲, Hewitt et al. (69).*

to inactivate greater than 1 to 3 log units of HAV or poliovirus. It is also important to note that these are extrapolated values and that use of different foods and/or heating conditions may result in altered heating characteristics.

There are apparently no studies on thermal inactivation of human noroviruses in dairy products. Thus, no comparisons were made between human noroviruses and cultivable surrogates. For future studies, the evaluation of thermal inactivation behavior of human norovirus in dairy products should be encouraged in order to learn more about the thermal resistance of these viruses.

Meat products. Very limited information about the thermal inactivation behavior of human norovirus surrogates and HAV in meat products is available, with only one study on turkey deli meat (Table 7). The calculated *D*-values (50 to 72°C) ranged from 0.14 to 9.94 min for FCV-F9, 0.22 to 21.01 min for MNV-1, and 1.01 to 42.08 min for HAV *(21)* (Table 7). The *z*-values for FCV-F9, MNV-1, and HAV were 11.90, 10.91, and 12.83°C, respectively *(21)*. In general, HAV was more resistant to thermal treatment than was FCV-F9 or MNV-1 at all temperatures studied, suggesting that it would require a more severe treatment than that used for human norovirus surrogates for inactivation of HAV in turkey deli meat. An industrial pasteurization process time for turkey deli meat could be estimated; treatment times of 107, 46, 20, 9, and 4 s would be required to achieve 6-log reductions of HAV in turkey deli meat at 80, 85, 90, 95, and 100°C, respectively. These processes would of course require heat penetration experiments.

Factors affecting the efficiency of thermal treatment. The apparent thermal resistance of viruses is specific for the heating conditions utilized and the conditions of detection. Factors influencing heat resistance can be divided into inherent, environmental (including heating system), and intrinsic (associated with the food). Inherent factors are associated with the viruses, i.e., the type of virus, the presence or absence of an envelope, the type of genome (e.g., DNA, RNA, single stranded, or double stranded), and the method of replication.

The apparent thermal resistance may vary depending upon intrinsic characteristics of the food in which the virus is found. During thermal treatment, the composition of the surrounding medium or food (e.g., presence of proteins, carbohydrates, fats, or salt) used in the heating process may influence the apparent thermal resistance. Thermal inactivation data available in the literature revealed that the apparent thermal resistance of foodborne enteric viruses was highly dependent on the food matrix, as there were significant differences among food types and between food and cell culture media. Differences in inactivation between different food matrices may be due to compositional differences of food samples (e.g., seafood; dairy products; fruits, vegetables, and herbs; and meat products) because the environment in which viruses are found influences their sensitivity to thermal inactivation. It has been reported that the presence of certain food components, such as protein and fat, may

TABLE 7. *Thermal inactivation of foodborne enteric viruses and their viral surrogates in meat products*

Virus	Enumeration unit	Sample	Temp (°C)	*D*-value (min)	R^2	*z*-value (°C)	R^2	Reference
Feline calicivirus (FCV-F9)	PFU/ml	Turkey deli meat	50	9.94	0.94	10.91	0.98	*21*
			56	3.03	0.94			
			60	0.82	0.96			
			65	0.43	0.89			
			72	0.14	0.90			
Murine norovirus (MNV-1)	PFU/ml	Turkey deli meat	50	21.01	0.97	12.83	0.99	*21*
			56	7.3	0.99			
			60	2.74	0.98			
			65	0.94	0.94			
			72	0.22	0.96			
Hepatitis A virus (HAV)	PFU/ml	Turkey deli meat	50	42.08	0.97	11.90	0.97	*21*
			56	20.62	0.96			
			60	5.91	0.94			
			65	2.27	0.95			
			72	1.01	0.93			

play a protective role against heat inactivation of foodborne enteric viruses *(12, 42, 43, 97, 104)*. Bidawid et al. *(12)* investigated the effect of fat concentration (1, 3.5, and 18.5%) on the heat resistance of HAV in milk and concluded that higher fat concentrations played a protective role and increased the stability of viruses. This finding was in agreement with that of Parry and Mortimer *(104),* who found similar protective effects for poliovirus inactivation in milk. It has been suggested that the presence of fat and protein in the heating medium influences the heat inactivation rate by protecting the cell receptors by formation of viral aggregates *(43)*.

Another potential factor associated with the heating condition is heat transfer rate, which is a function of the food product and the container size and shape. For example, container size causes differences in the heat transfer rate and thus affects the time to reach the desired temperature, which in turn affects apparent resistance *(38).* Therefore, the inclusion of come-up time during process time calculations is important. The contribution of sample size to apparent thermal resistance of human norovirus surrogates (FCV-F9 and MNV-1) was investigated by Bozkurt et al. *(17),* who reported that, especially at high temperatures, an increase in container size contributed to differences in the *D*-value due to increased come-up times. Hence, the reduction in the number of survivors during the come-up time is important to determine precise thermal process conditions.

The type of heating system could also influence the heating of the product and thus the heat transfer rate. Various methods for heat treatment have been used in the studies included in this review. A preponderance of the reported experiments have been done in a controlled temperature water bath. The exceptions were a boiling water bath *(68, 97, 115),* a gas-powered steam oven *(31),* a conventional oven *(27),* and a glycerol bath *(48)*. Different heating systems might result in different heating characteristics. In the oven method with clams, heat transfer occurs by convection between the heating medium and the food sample, and then heat transfer takes place by conduction throughout the sample. Therefore, a temperature gradient was observed throughout the clams during the heat treatment. However, in water bath studies, both heat transfer by both conduction and convection takes place, and the temperature throughout the sample is more uniform. Since, the primary objective of inactivation studies is to investigate the interaction of the virus and heat, homogenized samples are useful for obtaining a uniform food matrix and a more even temperature distribution. To obtain good thermal inactivation data, it is important to use a method of heat treatment that avoids local temperature variations *(122)*.

Following heat treatment, the methods for recovery and detection are important in determining the actual number of surviving virions. Methods for recovery of viruses from foods have two basic steps: (i) sample treatment and virus extraction and (ii) detection assay. The sample treatment can itself be performed in four steps: (i) elution of viruses from the food to leave them in suspension, (ii) removal of food substances from the virus suspension, (iii) concentration of suspended viruses, and (iv) extraction of nucleic acids from the concentrated viruses (in the case of molecular detection). Current methods described for treatment of samples for virus detection require multiple steps, which often result in limited extraction efficiency. Thus, in the detection of viruses from foods, a major stumbling block is the high variation, both quantitative and qualitative, in the recovery of viruses from food products. Since the average infectious dose of human noroviruses or HAV is about 10 to 100 particles, effective testing methods need to successfully extract and detect very small levels of virus *(118)*.

After sample treatment, the detection assay of viruses is based on one of two main principles: (i) the detection of infectious viruses by propagation in cell culture or (ii) the detection of viral genomes by molecular amplification techniques such as RT-PCR or rRT-PCR. The detection by cell culture is mainly based on cytopathic effects, followed by quantification of the viruses using a plaque assay, the most probable number, or the $TCID_{50}$.

The theoretical relationship between the $TCID_{50}$ and number of PFUs is approximately 0.69 PFU = 1 $TCID_{50}$ based on the Poisson distribution *(100)*. However, it must be emphasized that in practice, this relationship may not hold even for the same virus and cell combination because the two types of assays are set up differently and virus infectivity is very sensitive to various factors such as cell age and overlay medium *(32)*. In comparison to the $TCID_{50}$, the plaque assays also offer the specific advantage of a countable event, i.e., plaque formation *(45)*. In contrast, molecular detection methods are prone to inhibition and can produce false-negative results. Most false-negative results are a consequence of inefficient viral extraction or inhibition of the PCR. Most false-positive results are a consequence of cross-contamination. Therefore, the most recent methods include an internal amplification control, an external control for monitoring extraction efficiency, or both *(44)*. Also, specific primers are needed to prevent cross-reactivity from the food matrix *(16)*. To differentiate between infectious and noninfectious viruses by molecular methods, various pretreatment procedures and/or modifications of molecular methods (using viability dyes or binding to receptors) are being studied. Other recent studies and/or reviews have provided detailed information about current developments in molecular assays for human norovirus detection and determination of infectivity *(58, 84, 129)*.

MECHANISMS OF INACTIVATION OF VIRUSES DURING THERMAL TREATMENT

Foodborne enteric viruses are nonenveloped, positive-sense RNA viruses that are surrounded by a capsid formed by capsomers *(49)*. Since the virus capsid encloses the viral genome, which encodes components necessary for virus structure or function and is responsible for binding to the host, it is likely that the mechanism of thermal inactivation of viruses would be associated with changes in the virus capsid. Pollard *(105)* discussed the theory of virus inactivation during thermal treatment and concluded that structural alterations in viral proteins occur due to the differential expansion of the various parts of the virus under the action

of heat. Heat disrupts the hydrogen bonding and destroys the spatial relationships necessary to maintain the structural integrity of viral proteins. Pollard *(105)* also stated that it is quite possible that the various components of the virus, such as the capsid and nucleic acids, have widely different values of entropy and enthalpy and therefore the degradation rates of these components would be different. Similarly, Song et al. *(116)* concluded that the mechanisms of thermal inactivation included denaturation of viral proteins as well as destruction of virus particles into noninfectious viral subunits and single proteins and that the mode of action during thermal treatment depends on the temperature. At mild temperatures (<56°C), the destruction of the viral receptor and structural changes in the capsid might cause inactivation by disrupting the specific structures needed to recognize and bind the host cells *(132)*. This hypothesis is supported by findings that the quaternary structure of the capsid was unaffected up to 60°C *(5)*; however, above 60°C, an alteration of the tertiary protein structure occurs, which facilitates access of thermal energy to nucleic material. Therefore, the capsid ceases to play a protective role, and inactivation of nucleic material results *(81)*. Thus, the increased inactivation rate at higher temperatures (>65°C) could be due to changes in the tertiary structures of the virus *(5, 128)*.

CONCLUSIONS

The goal of this review was to examine the current literature on thermal inactivation of foodborne enteric viruses and viral surrogates. One thing that is very evident is that there are still many gaps in the knowledge base on viral inactivation by heat. In comparison to the historical research on the heat resistance of bacteria, the research on heat resistance of enteric viruses is in its infancy and is being done essentially in reverse. For bacteria, target pathogens were the first to have their thermal inactivation kinetics studied, followed by a search for nonpathogenic surrogates. For enteric viruses, because there is no simple method for studying the inactivation kinetics of the target pathogen, surrogates must be used. How close the thermal inactivation kinetics of the surrogates are to those of the pathogens remains somewhat speculative. Research with the surrogates is not without value; however, it must be presumed that the ability to determine the viability of the target, i.e., human norovirus, will be improved. Once that is done, the reliability of the inactivation kinetics can be improved, and the relative resistance between the target and the surrogates will be known. This information will make thermal resistance data for surrogates in foods very valuable.

Another thing that is apparent even with the gaps is that enteric viruses are more resistant to heat than are the most heat-resistant vegetative bacterial pathogens. Thus, current processing recommendations based on data for vegetative bacterial pathogens may not eliminate similar levels of foodborne enteric viruses. There is a definite need for rethinking of target pathogens when determining guidelines for thermal processes. Even though it may take time to establish the guidelines, we have to reconsider the basis for choosing thermal process conditions in order to reduce the risk of viral foodborne illness outbreaks.

Future research needs in this area are first to close some of the knowledge gaps, including continued research on the thermal inactivation kinetics of surrogates and human norovirus in food products that may be susceptible to contamination. After looking at the data presented here, it is obvious that there is a great deal of variability in the inactivation kinetics among studies, which is likely due to the variation in methods used. Thus, there also should be studies to determine the effect of different heating and recovery methods on kinetics so that better comparisons can be made, along with suggestions for universal protocols for virus recovery and detection in each food category. Finally, there is a definite need for research to validate some of the new detection assays that are designed to differentiate infectious viruses from inactivated viruses in order to correlate data on human noroviruses with infectivity of cultivable surrogates.

ACKNOWLEDGMENT

The authors gratefully acknowledge the funding for this research that was provided by the U.S. Department of Agriculture National Agriculture and Food Research Initiative Grant 2011-68003-20096.

REFERENCES

1. Abad, F. X., R. M. Pintó, and A. Bosch. 1994. Survival of enteric viruses on environmental fomites. *Appl. Environ. Microbiol.* 60:3704–3710.
2. Abad, F. X., C. Villena, S. Guix, S. Caballero, R. M. Pintó, and A. Bosch. 2001. Potential role of fomites in the vehicular transmission of human astroviruses. *Appl. Environ. Microbiol.* 67:3904–3907.
3. Adams, M. R. and M. O. Moss. 2000. Food microbiology. Royal Society of Chemistry, Cambridge.
4. Arauz-Ruiz, P., L. Sundqvist, Z. Garcia, L. Taylor, K. Visona, H. Norder, and L. O. Magnius. 2001. Presumed common source outbreaks of hepatitis A in an endemic area confirmed by limited sequencing within the VP1 region. *J. Med. Virol.* 65:449–456.
5. Ausar, S. F., T. R. Foubert, M. H. Hudson, T. S. Vedvick, and C. R. Middaugh. 2006. Conformational stability and disassembly of Norwalk virus–like particles: effect of pH and temperature. *J. Biol. Chem.* 281:19478–19488.
6. Bae, J., and K. J. Schwab. 2008. Evaluation of murine norovirus, feline calicivirus, poliovirus, and MS2 as surrogates for human norovirus in a model of viral persistence in surface water and groundwater. *Appl. Environ. Microbiol.* 74:477–484.
7. Baert, L., M. Uyttendaele, E. Van Coillie, and J. Debevere. 2008. The reduction of murine norovirus 1, *B. fragilis* HSP40 infecting phage B40-8 and E. coli after a mild thermal pasteurization process of raspberry puree. *Food Microbiol.* 25:871–874.
8. Baert, L., M. Uyttendaele, M. Vermeersch, E. Van Coillie, and J. Debevere. 2008. Survival and transfer of murine norovirus 1, a surrogate for human noroviruses, during the production process of deep-frozen onions and spinach. *J. Food Prot.* 71:1590–1597.
9. Beller, M., A. Ellis, S. H. Lee, M. A. Drebot, S. A. Jenkerson, E. Funk, M. D. Sobsey, O. D. Simmons, S. S. Monroe, T. Ando, J. Noel, M. Petric, J. P. Middaugh, and J. S. Spika. 1997. Outbreak of viral gastroenteritis due to a contaminated well—international consequences. *JAMA (J. Am. Med. Assoc.)* 278:563–568.
10. Bertolotti-Ciarlet, A., S. E. Crawford, A. M. Hutson, and M. K. Estes. 2003. The 3′ end of Norwalk virus mRNA contains determinants that regulate the expression and stability of the viral capsid protein VP1: a novel function for the VP2 protein. *J. Virol.* 77:11603–11615.

11. Bertrand, I., J. F. Schijven, G. Sanchez, P. Wyn-Jones, J. Ottoson, T. Morin, M. Muscillo, M. Verani, A. Nasser, A. M. de Roda Husman, M. Myrmel, J. Sellwood, N. Cook, and C. Gantzer. 2012. The impact of temperature on the inactivation of enteric viruses in food and water: a review. *J. Appl. Microbiol.* 112:1059–1074.
12. Bidawid, S., J. M. Farber, S. A. Sattar, and S. Hayward. 2000. Heat inactivation of hepatitis A virus in dairy foods. *J. Food Prot.* 63:522–528.
13. Black, E. P., J. Cascarino, D. Guan, K. E. Kniel, D. T. Hicks, L. F. Pivarnick, and D. G. Hoover. 2010. Coliphage as pressure surrogates for enteric viruses in foods. *Innov. Food Sci. Emerg. Technol.* 11:239–244.
14. Blanton, L. H., S. M. Adams, R. S. Beard, G. Wei, S. N. Bulens, M. A. Widdowson, R. I. Glass, and S. S. Monroe. 2006. Molecular and epidemiologic trends of caliciviruses associated with outbreaks of acute gastroenteritis in the United States, 2000–2004. *J. Infect. Dis.* 193:413–421.
15. Bonaccio, M., A. Credali, and A. Peracchi. 2004. Kinetic and thermodynamic characterization of the RNA-cleaving 8-17 deoxyribozyme. *Nucleic Acids Res.* 32:916–925.
16. Bosch, A., G. Sánchez, M. Abbaszadegan, A. Carducci, S. Guix, F. S. Le Guyader, R. Netshikweta, R. M. Pintó, W. H. M. van der Poel, S. Rutjes, D. Sano, M. B. Taylor, W. B. van Zyl, D. Rodríguez-Lázaro, K. Kovač, and J. Sellwood. 2011. Analytical methods for virus detection in water and food. *Food Anal. Methods* 4:4–12.
17. Bozkurt, H., D. H. D'Souza, and P. M. Davidson. 2013. Determination of the thermal inactivation kinetics of the human norovirus surrogates, murine norovirus and feline calicivirus. *J. Food Prot.* 76:79–84.
18. Bozkurt, H., D. H. D'Souza, and P. M. Davidson. 2014. A comparison of the thermal inactivation kinetics of human norovirus surrogates and hepatitis A virus in buffered cell culture medium. *Food Microbiol.* 42:212–217.
19. Bozkurt, H., D. H. D'Souza, and P. M. Davidson. 2014. Determination of thermal inactivation kinetics of hepatitis A virus in blue mussel (*Mytilus edulis*) homogenate. *Appl. Environ. Microbiol.* 80:3191–3197.
20. Bozkurt, H., D. H. D'Souza, and P. M. Davidson. 2014. Thermal inactivation of human norovirus surrogates in spinach and measurement of its uncertainty. *J. Food Prot.* 77:276–283.
21. Bozkurt, H., D. H. D'Souza, and P. M. Davidson. Determination of thermal inactivation kinetics of human norovirus surrogates and hepatitis A virus in turkey deli meat. *Appl. Environ. Microbiol.,* in press.
22. Bozkurt, H., S. Leiser, P. M. Davidson, and D. H. D'Souza. 2014. Thermal inactivation kinetic modeling of human norovirus surrogates in blue mussel (*Mytilus edulis*) homogenate. *Int. J. Food Microbiol.* 172:130–136.
23. Bozkurt, H., X. Ye, F. M. Harte, D. H. D'Souza, and P. M. Davidson. 2015. Thermal inactivation kinetics of hepatitis A virus in spinach. *Int. J. Food Microbiol.* 193:147–151.
24. Brundage, S. C., and A. N. Fitzpatrick. 2006. Hepatitis A. *Am. Fam. Physician* 73:2162–2168.
25. Buckow, R., S. Isbarn, D. Knorr, V. Heinz, and A. Lehmacher. 2008. Predictive model for inactivation of feline calicivirus, a norovirus surrogate, by heat and high hydrostatic pressure. *Appl. Environ. Microbiol.* 74:1030–1038.
26. Busta, F. F., T. V. Suslow, M. E. Parish, L. F. Beuchat, J. N. Farber, E. H. Garrett, and L. H. Harris. 2003. The use of indicators and surrogate microorganisms for the evaluation of pathogens in fresh and fresh-cut produce. *Compr. Rev. Food Sci. Food Saf.* 2:179–185.
27. Butot, S., T. Putallaz, R. Amoroso, and G. Sanchez. 2009. Inactivation of enteric viruses in minimally processed berries and herbs. *Appl. Environ. Microbiol.* 75:4155–4161.
28. Caballero, S., F. X. Abad, F. Loisy, F. S. Le Guyader, J. Cohen, R. M. Pintó, and A. Bosch. 2004. Rotavirus virus-like particles as surrogates in environmental persistence and inactivation studies. *Appl. Environ. Microbiol.* 70:3904–3909.
29. Calender, R. 1988. The bacteriophages, vol. I. Plenum Press, New York.
30. Cannon, J. L., E. Papafragkou, G. W. Park, J. Osborne, L. A. Jaykus, and J. Vinjé. 2006. Surrogates for the study of norovirus stability and inactivation in the environment: a comparison of murine norovirus and feline calicivirus. *J. Food Prot.* 69:2761–2765.
31. Cappellozza, E., G. Arcangeli, M. Rosteghin, S. Kapllan, C. Magnabosco, E. Bertoli, and C. Terregino. 2012. Survival of hepatitis A virus in pasteurized Manila clams. *Ital. J. Food Sci.* 24: 247–253.
32. Carter, J. C., and V. A. Saunders. 2007. Virology: principles and applications. John Wiley & Sons, Chichester, UK.
33. Centers for Disease Control and Prevention. 2014. 2011 Estimates of foodborne illnesses in the United States. Available at: http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html. Accessed 12 February 2014.
34. Centers for Disease Control and Prevention. 2014. Hepatitis A FAQs for health professional. Available at: http://www.cdc.gov/hepatitis/HCV/HCVfaq.htm. Accessed 12 February 2014.
35. Centers for Disease Control and Prevention. 2014. A–Z index for foodborne illnesses. Available at: http://search.cdc.gov/search?query=a-z+index+for+foodborne+illness&searchButton.x=0&searchButton.y=0&action=search&utf8=%E2%9C%93&affiliate=cdc-main. Accessed 12 February 2014.
36. Chan, M. C., J. J. Sung, R. K. Lam, P. K. Chan, N. L. Lee, R. W. Lai, and W. K. Leung. 2006. Fecal viral load and norovirus-associated gastroenteritis. *Emerg. Infect. Dis.* 12:1278–1280.
37. Chen, J., W. X. Li, D. Xie, J. R. Peng, and S. W. Ding. 2004. Viral virulence protein suppresses RNA silencing–mediated defense but upregulates the role of microRNA in host gene expression. *Plant Cell* 16:1302–1313.
38. Chung, H. J., S. J. Wang, and J. M. Tang. 2007. Influence of heat transfer with tube methods on measured thermal inactivation parameters for *Escherichia coli*. *J. Food Prot.* 70:851–859.
39. Clark, I. N., M. K. Estes, K. Y. Green, G. S. Hansman, N. J. Knowles, M. K. Koopmans, D. O. Matson, G. Meyers, J. D. Neill, A. Radford, A. W. Smith, M. J. Studdert, H. J. Thiel, and J. Vinjé. 2012. Family Caliciviridae, p. 977–986. *In* A. M. Q. King, M. J. Adams, E. B. Carstens, and E. J. Lefkowitz (ed.), Virus taxonomy: classification and nomenclature of viruses. Ninth report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, London.
40. Croci, L., M. Ciccozzi, D. De Medici, S. Di Pasquale, A. Fiore, A. Mele, and L. Toti. 1999. Inactivation of hepatitis A virus in heat-treated mussels. *J. Appl. Microbiol.* 87:884–888.
41. Croci, L., D. De Medici, S. Di Pasquale, and L. Toti. 2005. Resistance of hepatitis A virus in mussels subjected to different domestic cookings. *Int. J. Food Microbiol.* 105:139–144.
42. Croci, L., D. De Medici, G. Morace, A. Fiore, C. Scalfaro, F. Beneduce, and L. Toti. 1999. Detection of hepatitis A virus in shellfish by nested reverse transcription–PCR. *Int. J. Food Microbiol.* 48:67–71.
43. Croci, L., E. Suffredini, S. Di Pasquale, and L. Cozzi. 2012. Detection of norovirus and feline calicivirus in spiked molluscs subjected to heat treatments. *Food Control* 25:17–22.
44. D'Agostino, M., C. N., D. Rodriguez-Lazaro, and S. Rutjes. 2011. Nucleic acid amplification–based methods for detection of enteric viruses: definition of controls and interpretation of results. *Food Environ. Virol.* 3:55–60.
45. Darling, A. J., J. A. Boose, and J. Spaltro. 1998. Virus assay methods: accuracy and validation. *Biologicals* 26:105–110.
46. Dawson, D. J., A. Paish, L. M. Staffell, I. J. Seymour, and H. Appleton. 2005. Survival of viruses on fresh produce, using MS2 as a surrogate for norovirus. *J. Appl. Microbiol.* 98:203–209.
47. Debbink, K., L. C. Lindesmith, and R. S. Baric. 2014. The state of norovirus vaccines. *Clin. Infect. Dis.* 58:1746–1752.
48. Deboosere, N., O. Legeay, Y. Caudrelier, and M. Lange. 2004. Modelling effect of physical and chemical parameters on heat inactivation kinetics of hepatitis A virus in a fruit model system. *Int. J. Food Microbiol.* 93:73–85.

49. Dimmock, N. J., A. J. Easton, and K. N. Leppard. 2001. Introduction to modern virology, 5th ed. Blackwell Publishing, Malden, MA.
50. Donaldson, E. F., L. C. Lindesmith, A. D. Lobue, and R. S. Baric. 2008. Norovirus pathogenesis: mechanisms of persistence and immune evasion in human populations. *Immunol. Rev.* 225:190–211.
51. Doultree, J. C., J. D. Druce, C. J. Birch, D. S. Bowden, and J. A. Marshall. 1999. Inactivation of feline calicivirus, a Norwalk virus surrogate. *J. Hosp. Infect.* 41:51–57.
52. Dowell, S. F., C. Groves, K. B. Kirkland, H. G. Cicirello, T. Ando, Q. Jin, J. R. Gentsch, S. S. Monroe, C. D. Humphrey, C. Slemp, D. M. Dwyer, R. A. Meriwether, and R. I. Glass. 1995. A multistate outbreak of oyster-associated gastroenteritis—implications for interstate tracing of contaminated shellfish. *J. Infect. Dis.* 171:1497–1503.
53. D'Souza, D. H., K. Kniel, and L. A. Jaykus. 2011. Hepatitis A virus in ready-to-eat foods, p. 393–412. *In* J. Hoorfar (ed.), Rapid detection, characterization, and enumeration of foodborne pathogens. ASM Press, Washington, DC.
54. D'Souza, D. H., C. L. Moe, and L. A. Jaykus. 2007. Foodborne viral pathogens, p. 581–607. *In* M. P. Doyle and L. R. Beuchat (ed.), Food microbiology: fundamentals and frontiers. ASM Press, Washington, DC.
55. D'Souza, D. H., A. Sair, K. Williams, E. Papafragkou, J. Jean, C. Moore, and L. Jaykus. 2006. Persistence of caliciviruses on environmental surfaces and their transfer to food. *Int. J. Food Microbiol.* 108:84–91.
56. Duizer, E., P. Bijkerk, B. Rockx, A. De Groot, F. Twisk, and M. Koopmans. 2004. Inactivation of caliciviruses. *Appl. Environ. Microbiol.* 70:4538–4543.
57. Duizer, E., Y. van Duynhoven, H. Vennema, and M. Koopmans. 2004. Failure to detect norovirus in a large group of asymptomatic individuals by Marshall et al. (Public Health Vol 118 (3) 230–233). *Public Health* 118:455–456; author reply 456–457.
58. Escudero-Abarca, B. I., H. Rawsthorne, R. M. Goulter, S. H. Suh, and L. A. Jaykus. 2014. Molecular methods used to estimate thermal inactivation of a prototype human norovirus: more heat resistant than previously believed? *Food Microbiol.* 41:91–95.
59. Farkas, T., R. W. Cross, E. Hargitt III, N. W. Lerche, A. L. Morrow, and K. Sestak. 2010. Genetic diversity and histo-blood group antigen interactions of rhesus enteric caliciviruses. *J. Virol.* 84:8617–8625.
60. Farkas, T., K. Sestak, C. Wei, and X. Jiang. 2008. Characterization of a rhesus monkey calicivirus representing a new genus of Caliciviridae. *J. Virol.* 82:5408–5416.
61. Flynn, W. T., L. J. Saif, and P. D. Moorhead. 1988. Pathogenesis of porcine enteric calicivirus-like virus in four-day-old gnotobiotic pigs. *Am. J. Vet. Res.* 49:819–825.
62. Gibson, K. E., and K. J. Schwab. 2011. Thermal inactivation of human norovirus surrogates. *Food Environ. Virol.* 3:74–77.
63. Green, K. Y., R. M. Chanock, and A. Z. Kapikian. 2001. Human caliciviruses, p. 841–874. *In* D. M. Knipe and P. M. Howley (ed.), Fields virology, 4th ed. Lippincott-Raven, Philadelphia.
64. Grove, S. F., A. Lee, T. Lewis, C. M. Stewart, H. Q. Chen, and D. G. Hoover. 2006. Inactivation of foodborne viruses of significance by high pressure and other processes. *J. Food Prot.* 69:957–968.
65. Hall, A. J., M. E. Wikswo, K. Pringle, L. H. Gould, and U. D. Parashar. 2014. Vital signs: foodborne norovirus outbreaks—United States, 2009–2012. *Morb. Mortal. Wkly. Rep.* 63:491–495.
66. Heldman, D. R. 2011. Food preservation process design. Elsevier, Burlington, MA.
67. Herbst-Kralovetz, M. M., A. L. Radtke, M. K. Lay, B. E. Hjelm, A. N. Bolick, S. S. Sarker, R. L. Atmar, D. H. Kingsley, C. J. Arntzen, M. K. Estes, and C. A. Nickerson. 2013. Lack of norovirus replication and histo-blood group antigen expression in 3-dimensional intestinal epithelial cells. *Emerg. Infect. Dis.* 19:431–438.
68. Hewitt, J., and G. E. Greening. 2006. Effect of heat treatment on hepatitis A virus and norovirus in New Zealand greenshell mussels (*Perna canaliculus*) by quantitative real-time reverse transcription PCR and cell culture. *J. Food Prot.* 69:2217–2223.
69. Hewitt, J., M. Rivera-Aban, and G. E. Greening. 2009. Evaluation of murine norovirus as a surrogate for human norovirus and hepatitis A virus in heat inactivation studies. *J. Appl. Microbiol.* 107:65–71.
70. Hirneisen, K. A., E. P. Black, J. L. Cascarino, V. R. Fino, D. G. Hoover, and K. E. Kniel. 2010. Viral inactivation in foods: a review of traditional and novel food-processing technologies. *Compr. Rev. Food Sci. Food Saf.* 9:3–20.
71. Hirneisen, K. A., and K. E. Kniel. 2013. Comparing human norovirus surrogates: murine norovirus and Tulane virus. *J. Food Prot.* 76:139–143.
72. Hoeltzer, K., W. Fanaselle, R. Pouillot, J. M. van Doren, and S. Dennis. 2013. Virus inactivation on hard surfaces or in suspension by chemical disinfectants: systematic review and meta-analysis of norovirus surrogates. *J. Food Prot.* 76:1006–1016.
73. Hollinger, F. B., and S. U. Emerson. 5th ed., 2007. Hepatitis A virus, p. 911–940. *In* D. M. Knipe and P. M. Howley (ed.), Fields virology, 5th ed. Lippincott Williams & Wilkins, Philadelphia.
74. Houben, J. H., and F. Eckenhausen. 2006. Surface pasteurization of vacuum-sealed precooked ready-to-eat meat products. *J. Food Prot.* 69:459–468.
75. Huang, P. W., T. Farkas, W. M. Zhong, S. Thornton, A. L. Morrow, and J. Xi. 2005. Norovirus and histo-blood group antigens: demonstration of a wide spectrum of strain specificities and classification of two major binding groups among multiple binding patterns. *J. Virol.* 79:6714–6722.
76. Hutson, A. M., R. L. Atmar, and M. K. Estes. 2004. Norovirus disease: changing epidemiology and host susceptibility factors. *Trends Microbiol.* 12:279–287.
77. Jaykus, L. 2000. Enteric viruses as 'emerging agents' of foodborne disease. *Irish J. Agric. Food Res.* 39:245–255.
78. Jeong, S. H., and H. S. Lee. 2010. Hepatitis A: clinical manifestations and management. *Intervirology* 53:15–19.
79. Jones, M. K., M. Watanabe, S. Zhu, C. L. Graves, L. R. Keyes, K. R. Grau, M. B. Gonzalez-Hernandez, N. M. Iovine, C. E. Wobus, J. Vinjé, S. A. Tibbetts, S. M. Wallet, and S. M. Karst. 2014. Enteric bacteria promote human and mouse norovirus infection of B cells. *Science* 346:755–759.
80. Karst, S. M., C. E. Wobus, I. Goodfellow, K. Y. Green, and H. W. Virgin. 2014. Advances in norovirus biology. *Cell Host Microbe* 15:668–680.
81. Katen, S. P., Z. Tan, S. R. Chirapu, M. G. Finn, and A. Zlotnick. 2013. Assembly-directed antivirals differentially bind quasiequivalent pockets to modify hepatitis B virus capsid tertiary and quaternary structure. *Structure* 21:1406–1416.
82. Kaufman, S. S., K. Y. Green, and B. E. Korba. 2014. Treatment of norovirus infections: moving antivirals from the bench to the bedside. *Antiviral Res.* 105C:80–91.
83. Kingsley, D. H., D. G. Hoover, E. Papafragkou, and G. P. Richards. 2002. Inactivation of hepatitis A virus and a calicivirus by high hydrostatic pressure. *J. Food Prot.* 65:1605–1609.
84. Knight, A., D. Li, M. Uyttendaele, and L. A. Jaykus. 2013. A critical review of methods for detecting human noroviruses and predicting their infectivity. *Crit. Rev. Microbiol.* 39:295–309.
85. Kokkinos, P., P. Ziros, S. Filippidou, I. Mpampounakis, and A. Vantarakis. 2010. Molecular characterization of hepatitis A virus isolates from environmental and clinical samples in Greece. *Virol. J.* 7:235.
86. Koopmans, M., and E. Duizer. 2004. Foodborne viruses: an emerging problem. *Int. J. Food Microbiol.* 90:23–41.
87. Kukkula, M., L. Maunula, E. Silvennoinen, and C. H. von Bonsdorff. 1999. Outbreak of viral gastroenteritis due to drinking water contaminated by Norwalk-like viruses. *J. Infect. Dis.* 180:1771–1776.
88. Kurdziel, A. S., N. Wilkinson, S. Langton, and N. Cook. 2001. Survival of poliovirus on soft fruit and salad vegetables. *J. Food Prot.* 64:706–709.
89. Lee, J., and G. Kaletunc. 2002. Calorimetric determination of inactivation parameters of micro-organisms. *J. Appl. Microbiol.* 93:178–189.
90. Le Guyader, F. S., J. C. Le Saux, K. Ambert-Balay, J. Krol, O. Serais, S. Parnaudeau, H. Giraudon, G. Delmas, M. Pommepuy, P. Pothier,

and R. L. Atmar. 2008. Aichi virus, norovirus, astrovirus, enterovirus, and rotavirus involved in clinical cases from a French oyster-related gastroenteritis outbreak. *J. Clin. Microbiol.* 46:4011–4017.
91. Loisy, F., R. L. Atmar, J. C. Le Saux, J. Cohen, M. P. Caprais, M. Pommepuy, and F. S. Le Guyader. 2005. Use of rotavirus virus-like particles as surrogates to evaluate virus persistence in shellfish. *Appl. Environ. Microbiol.* 71:6049–6053.
92. Madigan, M. T., J. M. Martinko, and J. Parker. 2000. Brock biology of microorganisms, 9th ed. Prentice-Hall, Upper Saddle River, NJ.
93. Mariam, T. W., and D. O. Cliver. 2000. Small round coliphages as surrogates for human viruses in process assessment. *Dairy Food Environ. Sanit.* 20s:684–689.
94. Martin, A., and S. M. Lemon. 2006. Hepatitis A virus: from discovery to vaccines. *Hepatology* 43:S164–S172.
95. Mbithi, J. N., V. S. Springthorpe, J. R. Boulet, and S. A. Sattar. 1992. Survival of hepatitis A virus on human hands and its transfer on contact with animate and inanimate surfaces. *J. Clin. Microbiol.* 30:757–763.
96. Mele, A., M. G. Rastelli, O. N. Gill, D. di Bisceglie, F. Rosmini, G. Pardelli, C. Valtriani, and P. Patriarchi. 1989. Recurrent epidemic hepatitis A associated with consumption of raw shellfish, probably controlled through public health measures. *Am. J. Epidemiol.* 130:540–546.
97. Millard, J., H. Appleton, and J. V. Parry. 1987. Studies on heat inactivation of hepatitis A virus with special reference to shellfish. Part 1. Procedures for infection and recovery of virus from laboratory-maintained cockles. *Epidemiol. Infect.* 98:397–414.
98. Nainan, O. V., G. Xia, G. Vaughan, and H. S. Margolis. 2006. Diagnosis of hepatitis A virus infection: a molecular approach. *Clin. Microbiol. Rev.* 19:63–79.
99. Newell, D. G., M. Koopmans, L. Verhoef, E. Duizer, A. Aidara-Kane, H. Sprong, M. Opsteegh, M. Langelaar, J. Threfall, F. Scheutz, J. van der Giessen, and H. Kruse. 2010. Food-borne diseases—the challenges of 20 years ago still persist while new ones continue to emerge. *Int. J. Food Microbiol.* 139(Suppl. 1):S3–S15.
100. O'Reilly, D. R., K. L. Miller, and V. A. Luckow. 1994. Baculovirus expression vectors: a laboratory manual, revised ed. Oxford University Press, New York.
101. Papafragkou, E., J. Hewitt, G. W. Park, G. Greening, and J. Vinjé. 2013. Challenges of culturing human norovirus in three-dimensional organoid intestinal cell culture models. *PLoS One* 8:e63485.
102. Park, S. Y., and S.-D. Ha. 2014. Thermal inactivation of hepatitis A virus in suspension and in dried mussels (*Mytilus edulis*). *Int. J. Food Sci. Technol.* 50:717–722.
103. Park, S. Y., S.-H. Kim, I.-S. Ju, J.-I. Cho, and S.-D. Ha. 2014. Thermal inactivation of murine norovirus-1 in suspension and in dried mussels (*Mytilus edulis*). *J. Food Saf.* 34:193–198.
104. Parry, J. V., and P. P. Mortimer. 1984. The heat sensitivity of hepatitis A virus determined by a simple tissue culture method. *J. Med. Virol.* 14:277–283.
105. Pollard, E. C. 1960. Theory of the physical means of the inactivation of viruses. *Ann. N. Y. Acad. Sci.* 83:654–660.
106. Reiner, P., M. Reinerova, and Z. Veselovska. 1992. Comparison of two defective hepatitis A virus strains adapted to cell cultures. *Acta Virol.* 36:245–252.
107. Richards, G. P. 2012. Critical review of norovirus surrogates in food safety research: rationale for considering volunteer studies. *Food Environ. Virol.* 4:6–13.
108. Robertson, B. H., R. W. Jansen, B. Khanna, A. Totsuka, O. V. Nainan, G. Siegl, A. Widell, H. S. Margolis, S. Isomura, K. Ito, T. Ishizu, Y Moritsugu, and S. M. Lemon. 1992. Genetic relatedness of hepatitis A virus strains recovered from different geographical regions. *J. Gen. Virol.* 73:1365–1377.
109. Sair, A. I., D. H. D'Souza, C. L. Moe, and L. A. Jaykus. 2002. Improved detection of human enteric viruses in foods by RT-PCR. *J. Virol. Methods* 100:57–69.
110. Sattar, S. A., T. Jason, S. Bidawid, and J. Farber. 2000. Foodborne spread of hepatitis A: recent studies on virus survival, transfer and inactivation. *Can. J. Infect. Dis.* 11:159–163.
111. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States—major pathogens. *Emerg. Infect. Dis.* 17:7–15.
112. Siebenga, J. J., P. Lemey, S. L. Kosakovsky Pond, A. Rambaut, H. Vennema, and M. Koopmans. 2010. Phylodynamic reconstruction reveals norovirus GII.4 epidemic expansions and their molecular determinants. *PLoS Pathog.* 6(5):e1000884.
113. Silva, F. V. M., and P. A. Gibbs. 2012. Thermal pasteurization requirements for the inactivation of Salmonella in foods. *Food Res. Int.* 45:695–699.
114. Singh, R. P. 2005. Food freezing, p. 53–69. *In* G. V. B. Cánovas (ed.), Food engineering. Encyclopedia of Life Support Systems Publishers, Oxford.
115. Slomka, M. J., and H. Appleton. 1998. Feline calicivirus as a model system for heat inactivation studies of small round structured viruses in shellfish. *Epidemiol. Infect.* 121:401–407.
116. Song, H. S., J. Li, S. Shi, L. Yan, H. Zhuang, and K. Li. 2010. Thermal stability and inactivation of hepatitis C virus grown in cell culture. *Virol. J.* 7:40.
117. Sow, H., M. Desbiens, R. Morales-Rayas, S. E. Ngazoa, and J. Jean. 2011. Heat inactivation of hepatitis A virus and a norovirus surrogate in soft-shell clams (*Mya arenaria*). *Foodborne Pathog. Dis.* 8:387–393.
118. Stals, A., L. Baert, E. Van Coillie, and M. Uyttendaele. 2012. Extraction of food-borne viruses from food samples: a review. *Int. J. Food Microbiol.* 153:1–9.
119. Straub, T. M., R. A. Bartholomew, C. O. Valdez, N. B. Valentine, A. Dohnalkova, R. M. Ozanich, C. J. Bruckner-Lea, and D. R. Call. 2011. Human norovirus infection of Caco-2 cells grown as a three-dimensional tissue structure. *J. Water Health* 9:225–240.
120. Straub, T. M., K. Honer zu Bentrup, P. Orosz-Coghlan, A. Dohnalkova, B. K. Mayer, R. A. Bartholomew, C. O. Valdez, C. J. Bruckner-Lea, C. P. Gerba, M. Abbaszadegan, and C. A. Nickerson. 2007. In vitro cell culture infectivity assay for human noroviruses. *Emerg. Infect. Dis.* 13:396–403.
121. Strazynski, M., J. Kramer, and B. Becker. 2002. Thermal inactivation of poliovirus type 1 in water, milk and yoghurt. *Int. J. Food Microbiol.* 74:73–78.
122. Stringer, S. C., S. M. George, and M. W. Peck. 2000. Thermal inactivation of *Escherichia coli* O157:H7. *J. Appl. Microbiol. Symp. Suppl.* 88:79S–89S.
123. Takanashi, S., L. J. Saif, J. H. Hughes, T. Meulia, K. Jung, K. A. Scheuer, and Q. Wang. 2014. Failure of propagation of human norovirus in intestinal epithelial cells with microvilli grown in three-dimensional cultures. *Arch. Virol.* 159:257–266.
124. Teunis, P. F. M., C. L. Moe, P. Liu, S. E. Miller, L. Lindesmith, R. S. Baric, J. Le Pendu, and R. L. Calderon. 2008. Norwalk virus: how infectious is it? *J. Med. Virol.* 80:1468–1476.
125. Tian, P., D. Yang, C. Quigley, M. Chou, and X. Jiang. 2013. Inactivation of the Tulane virus, a novel surrogate for the human norovirus. *J. Food Prot.* 76:712–718.
126. Treanor, J. J., R. L. Atmar, S. E. Frey, R. Gormley, W. H. Chen, J. Ferreira, R. Goodwin, A. Borkowski, R. Clemens, and P. M. Mendelman. 2014. A novel intramuscular bivalent norovirus virus-like particle vaccine candidate—reactogenicity, safety, and immunogenicity in a phase 1 trial in healthy adults. *J. Infect. Dis.* 210:1763–1771.
127. van Damme, P., J. Banatvala, O. Fay, S. Iwarson, B. McMahon, K. Van Herck, D. Shouval, P. Bonanni, B. Connor, G. Cooksley, G. Leroux-Roels, F. Von Sonnenburg, and the International Consensus Group on Hepatitis A Virus Immunity. 2003. Hepatitis A booster vaccination: is there a need? *Lancet* 362:1065–1071.
128. Volking, D. B., C. J. Burke, K. E. Marfia, C. B. Oswald, B. Wolanski, and C. R. Middaugh. 1997. Size and conformational stability of the hepatitis A virus used to prepare VAQTA, a highly purified inactivated vaccine. *J. Pharm. Sci.* 86:666–673.

129. Wang, D., and P. Tian. 2014. Inactivation conditions for human norovirus measured by an in situ capture–qRT-PCR method. *Int. J. Food Microbiol.* 172:76–82.
130. Wang, Q., Z. Zhang, and L. J. Saif. 2012. Stability of and attachment to lettuce by a culturable porcine sapovirus surrogate for human caliciviruses. *Appl. Environ. Microbiol.* 78:3932–3940.
131. Wei, C., T. Farkas, K. Sestak, and X. Jiang. 2008. Recovery of infectious virus by transfection of in vitro–generated RNA from Tulane calicivirus cDNA. *J. Virol.* 82:11429–11436.
132. Wigginton, K. R., B. M. Pecson, T. Sigstam, F. Bosshard, and T. Kohn. 2012. Virus inactivation mechanisms: impact of disinfectants on virus function and structural integrity. *Environ. Sci. Technol.* 46:12069–12078.
133. Wobus, C. E., L. B. Thackray, and H. W. Virgin. 20006. Murine norovirus: a model system to study norovirus biology and pathogenesis. *J. Virol.* 80:5104–5112.
134. Yamashita, T., M. Sugiyama, H. Tsuzuki, K. Sakae, Y. Suzuki, and Y. Miyazaki. 2000. Application of a reverse transcription–PCR for identification and differentiation of Aichi virus, a new member of the Picornavirus family associated with gastroenteritis in humans. *J. Clin. Microbiol.* 38:2955–2961.
135. Yoder, J., V. Roberts, G. F. Craun, V. Hill, L. A. Hicks, N. T. Alexander, V. Radke, R. L. Calderon, M. C. Hlavsa, M. J. Beach, and S. L. Roy. 2008. Surveillance for waterborne disease and outbreaks associated with drinking water and water not intended for drinking—United States, 2005–2006. *Morb. Mortal. Wkly. Rep. Surveill. Summ.* 57:39–62.
136. Zheng, D. P., T. Ando, R. L. Fankhauser, R. S. Beard, R. I. Glass, and S. S. Monroe. 2006. Norovirus classification and proposed strain nomenclature. *Virology* 346:312–323.