The impact of thermal pasteurization on viral load in human milk and other matrices: A rapid review

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Abstract

Holder pasteurization (62.5°C, 30 min) of human milk (HM) is thought to reduce the risk of transmitting viruses to an infant. Some viruses may be secreted into milk – others may be contaminants. The effect of thermal pasteurization on viruses in HM has yet to be rigorously reviewed. The objective of this study is to characterize the effect of commonly used pasteurization techniques on viruses in HM and non-HM matrices. Databases (MEDLINE, Embase, Web of Science) were searched from inception to April 20th, 2020 for primary research articles assessing the impact of pasteurization on viral load or detection of live virus. Reviews were excluded, as were studies lacking quantitative measurements or those assessing pasteurization as a component of a larger process. Overall, 65,131 reports were identified, and 108 studies included. Pasteurization of HM at a minimum temperature of 56°C-60°C is effective at reducing detectable live virus. In cell culture media or plasma, coronaviruses (e.g., SARS-CoV, SARS-CoV-2, MERS) are highly susceptible to heating at ≥56°C. Although pasteurization parameters and matrices reported vary, all viruses studied, with the exception of parvoviruses, were susceptible to thermal killing. Future research important for the study of novel viruses should standardize pasteurization protocols and should test viral inactivation using a human milk matrix.

Novelty bullets

- In all matrices, including human milk, pasteurization at temperatures of 62.5°C was generally sufficient to reduce surviving viral load by several logs or to below the limit of detection.

- Holder pasteurization (62.5°C, 30 min) of human donor milk should be sufficient to inactivate non-heat resistant viruses, including coronaviruses, if present.
Keywords: viral infectivity, viruses, Holder pasteurization, thermal pasteurization, human milk, donor milk, milk banking, SARS-CoV-2
Introduction

Since the emergence of SARS-CoV-2 in late 2019, ensuring that current high-quality screening, handling and pasteurization standards are sufficient for maintaining a safe supply of human donor milk has been an ongoing challenge for milk banks (Furlow 2020). Human donor milk is used as a bridge for hospitalized infants while their mother’s own milk supply is being established; among very low birth weight infants, the use of human donor milk instead of preterm formula as a bridge has been shown to reduce the incidence of necrotizing enterocolitis (Underwood 2013; Quigley et al. 2019). Milk banking associations, including the Human Milk Banking Association of North America and the European Milk Banking Association have responded to the pandemic by issuing new guidelines with respect to enhanced donor screening, including asking specific questions to assess the likelihood of a potential donor being infected with SARS-CoV-2 (“COVID-19: EMBA Position Statement” 2020; “Milk Banking and COVID-19” 2020). While all donor milk from non-profit milk banks in North America is pasteurized using the Holder method (62.5°C, 30 min) to inactivate potentially pathogenic bacteria and viruses, additional research is warranted to determine whether SARS-CoV-2, is inactivated by Holder pasteurization (Arslanoglu et al. 2010; Guidelines for the Establishment and Operation of a Donor Human Milk Bank 2018).

At present, the virome of human milk has been understudied. Few studies have investigated whether or not viruses that may cause disease in preterm infants are present in human milk (Mohandas and Pannaraj 2020). Viruses may be present in human milk as a result of secretion into the milk in the mammary tissue, notably, cytomegalovirus, human t-lymphocytic virus, and human immunodeficiency virus (HIV), or may be present as a contaminant from skin...
or respiratory droplets either in the milk or on the containers (Michie 2001). Regardless of origin, it is important to understand how viruses found in human milk respond to thermal pasteurization. To date, there has been no systematic review of the impact of thermal pasteurization on viral load or live virus detection in a human milk matrix or other non-human milk matrices. The primary aim of this review is to characterize studies conducted in human milk to determine how certain viral families that are either present in human milk, or used as surrogates, respond to thermal pasteurization as assessed by viral load or live virus detection. To expand the scope of viruses tested, the secondary objective is to summarize studies conducted in non-human milk matrices that have examined the effect of thermal pasteurization on any virus. This review also aims to compare viruses that have been assessed in studies using both human milk and non-human milk matrices to ascertain any trends in susceptibility to thermal pasteurization.

**Materials and methods**

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed in completion of this rapid review, except where indicated (Moher et al. 2009). This rapid review is in response to the COVID-19 pandemic.

**Search strategy and selection criteria**

References for this rapid review were identified through electronic searches of various online databases including MEDLINE, Embase and Web of Science, from database inception to April 20th, 2020, with the assistance of a research librarian. The search strategy focused on keywords to identify articles that assessed the effect of thermal pasteurization or heat inactivation, including Holder pasteurization, on the detection of live virus or viral load in human milk or other non-human milk matrices. The names of viral families, as per the current taxonomic classification, were included in the search as they may be present in human milk (by secretion or
contamination) or could be used as surrogate viruses to model highly pathogenic or non-culturable viruses (King et al. 2012).

The keywords and MeSH terms included for all database searches were intended to capture all relevant research with respect to thermal pasteurization of viruses in human milk, the primary outcome of this rapid review. To increase the scope, we supplemented the search to capture research articles that tested all matrices other than human milk. The search strategy is summarized in Table S1 and included three main ideas. The first concept included viral taxonomic families using keywords and MeSH terms based on the nomenclature suggested by the International Committee on Taxonomy (King et al. 2012). The second concept consisted of synonyms and phrases closely related to human milk (e.g. breast milk, donor milk etc.). Lastly, the final concept was thermal pasteurization and its synonyms (e.g., Holder pasteurization, heat etc.). Our initial search aimed to retrieve articles specific to human milk which was achieved by combining all three concepts; by only retaining the first and last concept, a second set of articles was retrieved that theoretically involved thermal pasteurization and viruses in all other matrices, including human milk. Grey literature was searched as per the previously published guidelines including from dissertations and google advanced search (Natal 2019). Articles resulting from those searches and relevant references cited in those articles were reviewed.

After duplicates were removed, titles, then abstracts were screened by a single reviewer. Primary research articles were included if they assessed the effect of Holder pasteurization (62.5°C - 63°C) or any other heat treatment on viral load or detection of live virus in human milk or other matrices. Eligible study designs included pre-post or longitudinal; in either design, the outcome,
detection of live virus or viral load, was assessed before and after pasteurization, or at discrete
time points during a given pasteurization process. Qualitative, observational and review studies
were excluded, in addition to experimental studies that did not assess viral load (quantitative) or
detectable live virus. Studies that investigated how thermal pasteurization and the addition of
matrix stabilizers, affects viral load or live virus detection were also excluded; the outcome of
these studies may be confounded by the fact that the integrity of viruses may be different as
certain stabilizers are added or removed. Studies were also excluded if thermal pasteurization
was tested in combination with other processing techniques, (e.g., irradiation, lyophilization
during the production of plasma concentrates), unless the study was appropriately controlled.
The primary rationale being that aspects of processing, other than heat, may also lead to the
inactivation of viruses. Reports on clinical trials or studies published in non-scientific journals
were not included. All studies irrespective of language or year published were included.

Multiple attempts were made to retrieve the full-text of all articles screened on the basis of title
and abstract including interlibrary loan and/or author follow-up. Data were extracted from
eligible full-text articles including viruses tested, matrix used, thermal pasteurization parameters
(temperature, time) and a measure of reduction in viral load/detectable virus. Included studies
were summarized after being dichotomized into two groups depending on whether detectable
live virus or viral load was tested in human milk or another matrix. To determine whether a
human milk matrix affected the results, a subanalysis was conducted on studies that tested the
same viruses in both human milk and non-human milk matrices. In this subanalysis, only studies
that assessed virus presences by plaque reduction assay or endpoint dilution (TCID$_{50}$) were
included. First, viruses that were tested in both groups were determined by cross-referencing;
relevant data (log-reduction, temperature, and duration of pasteurization) was then extracted and aggregated. Unless otherwise defined, complete inactivation is a concentration of virus that was below the lower detection limit of the assay. If multiple studies assessed the same virus, the pasteurization conditions used in the summary were matched as closely as possible to the data available in studies experimenting with human milk.

**Results**

**Study selection and characteristics**

The selection of studies is summarized in Figure 1. A total of \( n = 65,131 \) reports were identified and assessed for eligibility. This included 23,441 citations from MEDLINE, 34,479 citations from Embase, 7,200 records from Web of Science and 11 from manual searches. Altogether, \( n = 64,950 \) records were excluded on the basis of title and abstracts alone, encompassing articles that did not meet the inclusion criteria (\( n = 44,287 \) records) or duplicate records (\( n = 20,663 \)). After title and abstract screening, 181 reports remained for full-text review. Upon full-text review, 73 reports were excluded: 6 were duplicate records, 2 could not be retrieved and 65 did not meet inclusion criteria. Thus, 108 articles were included in the review and were organized according to the matrix used in testing the effect of pasteurization on viral load.

**Studies conducted in human milk**

First, we summarized 18 unique studies that used human milk as the matrix to test the effect of pasteurization on thirteen different viruses (Table 1). Most studies reported on viral addition experiments, while few studies subjected milk with endogenous virus to thermal pasteurization. Predominantly, the viruses tested were caspid enveloped and belonged to 8 different viral families including: caliciviridae, filoviridae, flaviviridae, herpesviridae, papillomaviridae, picornaviridae, retroviridae, and togaviridae. Cytomegalovirus and HIV were the most common
viruses studied with 8 and 7 articles respectively. To assess surviving virus concentration following pasteurization, plaque reduction assays and endpoint titration assays (TCID₅₀) were most frequently used, although some studies used immunofluorescence, reverse-transcriptase enzymatic assays and secreted embryonic alkaline phosphatase (SEAP) reporter assay.

Based on the literature reviewed, Holder pasteurization, defined as a temperature of 62.5°C - 63°C held for 30 minutes, resulted in complete inactivation of viruses in the herpesviridae family, including cytomegalovirus (Dworsky et al. 1982; Hamprecht et al. 2004; Donalisio et al. 2014); however, complete inactivation of herpes simplex virus did not occur, requiring a temperature of 100°C, 5 min (Welsh et al. 1979). In fact, for cytomegalovirus specifically, some studies reported complete inactivation at 60°C-63°C for 5 seconds to 30 minutes (Friis and Andersen 1982; Klotz et al. 2018; Maschmann et al. 2019). Similarly, retroviridae were susceptible to heating in a human milk matrix whereby complete inactivation was observed after pasteurization above 60°C, for a minimum of 5 seconds. In particular, flash heating and Holder pasteurization completely inactivated HIV-1 in human milk (Orloff et al. 1993; Israel-Ballard et al. 2007; Volk et al. 2010; Hoque et al. 2013); high temperature short time (72°C for 8 seconds) similarly yielded complete inactivation (>5.5-log reduction) (Terpstra et al. 2007). Holder pasteurization was found to inactivate (>5-log reduction) Ebola virus and Marburg virus of the filoviridae family, Zika virus (>6-log reduction) of the flaviviridae family, Semliki forest virus of the togaviridae family (4.2-log reduction) and human papillomavirus of the papillomaviridae family (Welsh et al. 1979; Hamilton Spence et al. 2017; Pfaender et al. 2017). Some non-Envelope members of the picornaviridae family were found to be more resistant to heating (Terpstra et al. 2007); flash heating (72 °C for 16 seconds) of hepatitis A virus and
porcine parovirus yielded a 2- or 0.5-log reduction in TCID\textsubscript{50}/mL respectively. Infectivity of coxsackievirus persisted after Holder pasteurization, although reduced by 3.6-log PFU/mL (Welsh et al. 1979).

**Studies conducted in non-human milk matrices**

Second, we summarized the remaining 90 unique studies that were identified during the literature review that assessed the effect of thermal pasteurization on viruses in a non-human milk matrix (Table 2). Cell culture media was the most prevalent matrix used in testing; other common matrices included bovine milk, bovine serum, human serum albumin, human plasma. In total, 21 unique families of viruses were tested including: adenoviridae, anelloviridae, birnaviridae, caliciviridae, circonviridae, coronaviridae, flaviviridae, hepadnaviridae, hepeviridae, herpesviridae, orthomyxoviridae, paramyxoviridae, paroviridae, picornaviridae, polymaviridae, poxviridae, reoviridae, retroviridae, rhabdoviridae, togaviridae. The majority of studies tested non-enveloped viruses in the families of picornaviridae (\(n=38\)), and caliciviridae (\(n=24\)), in addition to retroviridae (\(n=16\)).

Hepatitis A was the most commonly tested virus tested of the picornaviridae family and was seen to be particularly heat sensitive in a variety of matrices including bovine milk, cell culture media and soft-shell clams. For example, a minimum of a 4-log reduction in infectivity of Hepatitis A was observed after different thermal pasteurization parameters such as 60°C-65°C for 10-180 min (Croci et al. 1999; Bidawid et al. 2000; Gibson and Schwab 2011); to 72°C for 1-13 min (Bidawid et al. 2000; Araud et al. 2016), and to 90°C for 5 min (Sow et al. 2011). Murine norovirus, the most frequently tested virus of the caliciviridae family, was also observed to be sensitive to heat. A reduction in infectivity of greater than 5-log was observed at temperatures of
60°C - 67°C for 1-60 min (Gibson and Schwab 2011; Shao et al. 2018), >3.5-log reduction at 72°C for 1 min (Hewitt et al. 2009; Araud et al. 2016), and >5-log reduction at 85°C -90°C for 1-5 min (Sow et al. 2011; Park et al. 2014a). HIV-1 was the most commonly tested of the retroviridae and was also susceptible to heat-inactivation. Greater than 4-log reduction in TCID$_{50}$ was observed at 60°C-65°C for 10-15 min (Lelie et al. 1987; Gregersen et al. 1989); similar reductions were observed at 77°C-80°C after 0.25 seconds (Charm et al. 1992).

Notably, viruses in the coronaviridae family, SARS-CoV, and SARS-CoV-2, also show significant reductions in infectivity (>5-7-log reduction in TCID$_{50}$/mL) following pasteurization; complete inactivation was observed at temperatures between 56°C-60°C for a 5-60 min duration (Duan et al. 2003; Darnell et al. 2004; Yunoki et al. 2004; Kariwa et al. 2006). Other coronaviruses, including canine coronavirus, and MERS show sensitivities to heating in cell culture media, where a clinically relevant reduction in infectivity (>4.5 – 5.5 log TCID$_{50}$) is attainable upon heating at 65°C for 5-15 minutes (Pratelli 2008; Leclercq et al. 2014).

Furthermore, cytomegalovirus, a member of the herpesviridae family was completely inactivated at temperatures between 50°C-65°C for 15-30 min (Plummer and Lewis 1965; Lelie et al. 1987; Farmer et al. 1992; Mikawa et al. 2019).

**Viruses tested in human milk and other matrices**

Finally, the summary of the comparisons among viruses that were tested in both a human milk and a non-human milk matrix is shown in Table 3. Overall, the range of temperatures that yielded some degree of log reduction were consistent among viruses, irrespective of the matrix. Cytomegalovirus, for example, was a virus where there was good agreement among studies testing thermal pasteurization in either a human milk or a non-human milk matrix; inactivation was evident at temperatures between 50°C and 65°C for 10-30 min. Similarly consistent, porcine
parvovirus in the parvoviridae family was found to be heat resistant in either human milk or non-human milk matrices (Danner et al. 1999; Terpstra et al. 2007; Sauerbrei and Wutzler 2009). There were some differences in the time required for the log reduction in infectivity depending on matrix, but there were no discernable trends identified.

**Discussion**

Pasteurization is an essential part of human donor milk banking and is practiced worldwide to reduce or eliminate the risk of transmission of viruses that may be expressed in milk or may be found as a contaminant; Holder pasteurization (62.5°C, 30 min) is the most common method used (Guidelines for the Establishment and Operation of a Donor Human Milk Bank 2018). Our rapid review aimed to summarize the literature pertaining to the effect of thermal pasteurization on viral load and detectable live virus; in particular, research that has been conducted using a human milk matrix. Our rapid review also aimed to compare viruses that have been both tested in a human milk matrix and a non-human milk matrix to better understand any potential modulating effects.

As expected, the most commonly studied viruses in human milk in relation to thermal pasteurization included those that have been previously shown to be transmitted through breastmilk; primarily cytomegalovirus and HIV-1 which are enveloped viruses belonging to the herpesviridae and retroviridae families respectively (Prendergast et al. 2019). Although not as common as cytomegalovirus or HIV, Ebola, Marburg, and Zika viruses have also been studied in human milk given that viral nucleic acid has been detected in milk and transmission is a potential concern (Hamilton Spence et al. 2017; Sampieri and Montero 2019). Despite differences in viral
taxonomy and caspid envelope, pasteurization is effective at significantly reducing detectable virus or viral load by several log, and in many cases, to below detectable levels (Table 1).

Many studies involving human milk tested pasteurization parameters that included the Holder method (62.5°C, 30 minutes) in order to mimic practices at milk banks; however, a variety of time and temperature combinations were tested. Although many studies reported that viruses including Ebola, Marburg, Zika, cytomegalovirus, and HIV appear to be completely inactivated after 30 min at 62.5°C - 63°C (Table 1), others report inactivation after a shorter duration; it remains unclear whether Holder pasteurization for shorter times might effectively inactivate these viruses. Arriving at a consensus is difficult given that one study might assess reductions in surviving virus concentrations before and after Holder pasteurization and another might assess at different time points during the pasteurization process. Moreover, high-temperature short-time pasteurization, defined here as pasteurization above 70°C for less than 30 minutes, appears to be as effective as pasteurization at lower temperatures for a longer duration.

Given the limited research in a human milk matrix, the inclusion of studies that assessed viral load or detectable live virus in a range of matrices allowed us to assess a broader scope of viruses belonging to numerous taxonomic families. The matrix may influence the effectiveness of pasteurization by altering how heat is distributed; however, our results suggest that irrespective of matrix, enveloped, compared to non-enveloped viruses, generally require less input of thermal energy in order to achieve similar reductions in viral load or live virus concentration. This suggests that the results presented in Table 2 may, to a certain degree, be representative of how viruses could be inactivated by heat in human milk. In all matrices, including human milk,
pasteurization at temperatures of 62.5°C was generally sufficient to reduce surviving viral load by several logs or to below the limit of detection—depending on the starting concentration of virus and whether it was enveloped. To completely inactivate non-enveloped viruses, such as bovine viral diarrhea virus, hepatitis A or porcine parvovirus in human milk or in other matrices, temperatures above 63°C (70°C -90°C) or a significantly longer duration at 60°C-63°C (Table 2) is generally required. Overall, the results are consistent with the logarithmic thermal death time curve where the same degree of thermal lethality can occur at varying temperatures depending on holding time; pasteurization at higher temperatures for shorter durations or lower temperatures for longer durations yielded similar results in terms of the magnitude of infectivity reductions.

Finally, while we cannot discount any differences in response to thermal pasteurization, viruses that were tested in both a human milk and non-human milk matrix appeared to require similar temperatures to elicit a given log reduction in infectivity. Nevertheless, there was significant variability in the duration of pasteurization tested, making it difficult to draw any conclusions as some viruses may require greater time at temperature for one matrix, and less time at temperature for another. In addition to there being a wide range of matrices included as part of the non-human milk group, differences in the time may be an artefact of the design of the respective studies; in many cases, viral infectivity or load was not always assessed longitudinally, but after a predetermined length of time. Consequently, this may overestimate the amount of time required to achieve a certain degree of inactivation, making it difficult to compare and aggregate the results from different studies.
There are many strengths of this rapid review. First, we carried out a robust search strategy, in addition to manual searches of grey literature, to generate a complete list of studies, irrespective of language or year published that assessed the impact of thermal pasteurization on viral load in human milk and other matrices. The studies in this review reported on a wide range of thermal pasteurization parameters (low-temperature long-time, high-temperature short-time) across several viruses in a diverse set of matrices. Despite these, the interpretation of our results should be considered alongside its limitations. First, this review was conducted by a single reviewer which may have introduced potential selection bias during initial screening. As a result, our review may not have captured all possible studies. Despite this, the purpose of this review was to rapidly and broadly characterize how viruses in any matrix, including human milk, might respond to thermal pasteurization. Second, the reduction in viral load or detectable live virus that was extracted was approximated if multiple strains of a given virus genus were studied, despite the potential of strain-specific variation in thermal resistance. Third, in our comparison of studies that assessed similar viruses in both a human milk and non-human milk matrix, we chose to aggregate the results to match, to the best of our ability, the pasteurization parameters tested in human milk. While this may have allowed us to assess the temperature and time requirements to achieve a certain log reduction, we were limited to a narrow range of pasteurization conditions.

To our knowledge, this rapid review is the first to broadly summarize the literature that has reported on the impact of any thermal pasteurization on virus survival. The results from this study highlight our limited understanding with respect to the effect of thermal pasteurization on viruses in human milk—this is especially relevant given the possibility that novel viruses, including SARS-CoV-2, may be present in human milk. Although currently, there is insufficient
evidence to suggest that SARS-CoV-2 is expressed in milk and could lead to vertical transmission, it may also be present as a contaminant (Lackey et al. 2020). Based on the literature review, Holder pasteurization (62.5°C, 30 minutes) may be sufficient to inactivate non-heat resistant viruses that may be present in HM, including coronaviruses. Though our attempt to rapidly survey all known viral families may help provide some insight into how novel viruses may respond to thermal pasteurization, additional research is warranted to synthesize empirical evidence using human milk as the matrix.

Acknowledgments:

This work was supported by the Ontario Graduate Scholarship; Restracomp Scholarship, The Hospital for Sick Children and the Canadian Institutes of Health Research [FDN# 143233]

The authors gratefully acknowledge Glyneva Bradley-Ridout at the University of Toronto who was consulted on the search strategy. All authors have no conflicts of interest to disclose.
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| Family       | Virus                   | Envelope | Pasteurization | Method          | Result                                                                 | Reference                      |
|-------------|-------------------------|----------|----------------|-----------------|------------------------------------------------------------------------|--------------------------------|
| Caliciviridae | Feline Calicivirus      | No       | 35°C-70°C, 2 min | PRA             | >4.5-log PFU/mL reduction at 60°C, 2min; >5-log PFU/mL reduction (complete inactivation) at 65°C, 2 min | Topping et al. 2009            |
| Filoviridae  | Ebola virus             | Yes      | 62.5°C, 30 min  | PRA             | >5-log PFU/mL reduction (complete inactivation) at 62.5°C, 30 min      | Hamilton Spence et al. 2017    |
|             | Marburg virus           | Yes      | 62.5°C, 30 min  | PRA             | >5-log PFU/mL reduction (complete inactivation) at 62.5°C, 30 min      | Hamilton Spence et al. 2017    |
| Flaviviridae | Bovine viral diarrhea virus | No     | 72°C, 16 sec    | TCID<sub>50</sub> | >6-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 72°C, 4 sec | Terpstra et al. 2007           |
|             | Zika virus              | Yes      | 63°C, 30 min    | TCID<sub>50</sub> | >4-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 60°C, 5s | Pfaender et al. 2017           |
| Herpesviridae| Cytomegalovirus         | Yes (1)  | 62°C, 2-15 sec; (2) 72°C, 5-15 sec; (3) 62°C, 30 min | Early antigen IF | Complete inactivation (no IEA + cells) for all treatments              | Klotz et al. 2018              |
|             | Cytomegalovirus         | Yes      | 62.5°C, 30 min  | SEAP Reporter   | Complete inactivation at 62.5°C, 30 min                                 | Donaldisio et al. 2014         |
|             | Cytomegalovirus         | Yes (1)  | 62.5°C, 30 min; (2) 72°C, 5 sec | Early Antigen IF | Complete inactivation (no IEA+ cells) at 62.5°C, 30 min or 72°C, 5 sec | Hamprecht et al. 2004          |
|             | Cytomegalovirus         | Yes      | (72°C, 87°C), 1-15 sec | PRA             | >5-log PFU/mL reduction (complete inactivation) at 72°C, 5 sec or 87°C, 5 sec | Goldblum et al. 1984           |
|             | Cytomegalovirus         | Yes      | 56°C, 62°C, 30 min | Cell culture toxicity | Complete inactivation (no cell culture toxicity) at 62°C, 30 min       | Dworsky et al. 1982            |
|             | Cytomegalovirus         | Yes      | 63°C, 1-16 min  | PRA             | 3.6-log PFU/mL reduction (complete inactivation) at 63°C, 8 min        | Friis and Andersen 1982        |
|             | Cytomegalovirus         | Yes (1)  | 56°C, 30 min; (2) 63°C, 30 min; (3) 100°C, 5 min | Cell culture toxicity | Complete inactivation (no detectable cytopathic effect) at 63°C, 30 min | Welsh et al. 1979              |
|             | Cytomegalovirus         | Yes (1)  | 56°C, 30 min; (2) 63°C, 30 min; (3) 100°C, 5 min | PRA             | 4.2-log PFU/mL reduction at 63°C, 30min; >7-log PFU/mL reduction (complete inactivation) at 100°C, 5 min | Welsh et al. 1979              |
|             | Pseudorabies virus      | Yes      | 72°C, 16 sec    | TCID<sub>50</sub> | >8-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 72°C, 4 sec | Terpstra et al. 2007           |
| Picornaviridae| Human papillomavirus    | No       | 62.5°C, 30 min  | SEAP Reporter   | Complete inactivation at 62.5°C, 30 min                                 | Donaldisio et al. 2014         |
| Parvoviridae | Porcine parovirus       | No       | 72°C, 16 sec    | TCID<sub>50</sub> | <1-log TCID<sub>50</sub>/mL reduction at 72°C, 16 sec                  | Terpstra et al. 2007           |
| Picornaviridae| Coxsackievirus B4       | No       | 63°C, 30 min    | PRA             | 3.8-log PFU/mL reduction at 56°C, 30 min; 3.6-log PFU/mL reduction at 63°C, 30 min; 7-log PFU/mL reduction at 100°C, 5 min | Welsh et al. 1979              |
| Picornaviridae| Hepatitis A virus       | No       | 72°C, 16 sec    | TCID<sub>50</sub> | 3.5-log TCID<sub>50</sub>/mL reduction at 72°C, 16 sec                 | Terpstra et al. 2007           |
| Retroviridae | HIV-1                   | Yes      | 54°C -57°C, 33 min | RT activity     | 4-log reduction (complete inactivation) at 56°C, 33 min                | Eglin, RP, Wilkinson 1987      |
| Retroviridae | HIV-1                   | Yes      | 55°C-70°C, time to max temperature | GFP indicator cells | 4-log IU/mL reduction (complete inactivation, no GFP+ cells) at 65°C, 5 sec | Hoque et al. 2013              |
| Viral Family | Virus | Complete Inactivation | Neutralization Assay | RT Activity | TCID<sub>50</sub> Activity | Notes |
|-------------|-------|-----------------------|----------------------|------------|-----------------------------|-------|
| Retroviridae | HIV-1 | Yes | 55°C-70°C, time to max temperature | PBMC neutralization assay | >3.4-log copies/mL reduction (complete inactivation) after flash heating | Complete inactivation after flash-heat treatment (Volk et al. 2010) |
| Retroviridae | HIV-1 | Yes | Flash heating (>56°C for 6 min (peak 73°C)) | RT activity | >8-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 72°C, 4 sec | (Israel-Ballard et al. 2007) |
| Retroviridae | HIV-1 | Yes | 72°C, 16 sec | TCID<sub>50</sub> | >5-log copies/mL reduction (complete inactivation) after pasteurization | (Terpstra et al. 2007) |
| Retroviridae | HIV-1 | Yes | 56°C-62.5°C, 12-15 min | RNA assay | >5.5-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 62.5°C, 30 min | (Jeffery et al. 2001) |
| Retroviridae | HIV-1 | Yes | 62.5°C, 30 min | TCID<sub>50</sub> | >4.2-log PFU/mL reduction at 63°C, 30 min >7-log PFU/mL reduction (complete inactivation) at 100°C, 5 min | (Orloff et al. 1993) |
| Togaviridae | Semliki forest virus | Yes | (1) 56°C, 30 min; (2) 63°C, 30 min; (3) 100°C, 5 min | PRA | >4.2-log PFU/mL reduction at 63°C, 30 min >7-log PFU/mL reduction (complete inactivation) at 100°C, 5 min | (Welsh et al. 1979) |

**Note.** Human immunodeficiency virus, HIV; Immunofluorescence, IF; Green fluorescence protein, GFP; plaque forming unit, PFU; Peripheral blood mononuclear cell, PBMC; Plaque reduction assay, PRA; Reverse transcriptase, RT; Secreted embryonic alkaline phosphatase, SEAP; Tissue culture infectious dose 50, TCID<sub>50</sub>. Complete inactivation refers to a viral load that is below the detectable limit of the assay, unless otherwise noted.
Table 2. Summary of studies assessing the effect of heat on viral inactivation in matrices others than human milk

| Family       | Virus                        | Envelope | Matrix               | Pasteurization | Method | Result                                                                 | Reference |
|--------------|------------------------------|----------|----------------------|----------------|--------|------------------------------------------------------------------------|-----------|
| Adenoviridae | Adenovirus type 12           | No       | Bovine milk          | 40°C-85°C, 0-30 min | PRA    | >3-log PFU/mL reduction in at 52°C, 40 min                             | (Sullivan et al. 1971) |
| Adenoviridae | Adenovirus type 5            | No       | Media                | 40°C-95°C, 1-2 h  | TCID₅₀  | >5.5-log TCID₅₀/mL reduction (complete inactivation) at 85°C, 2 h      | (Sauerbrei and Wutzler 2009) |
| Adenoviridae | Canine adenovirus            | No       | FBS                  | 56°C, 15-45 min  | TCID₅₀  | Complete inactivation (reduction factor ratio >6.58) at 56°C, 30 min  | (Danner et al. 1999) |
| Anelloviridae| Chicken anemia virus         | No       | Factor VIII          | 65°C-75°C, 30 min | TCID₅₀  | 0.91-log TCID₅₀/mL reduction at 65°C, 30 min; >3.5-log TCID₅₀/mL reduction at 75°C, 30 min; 4-log reduction PFU/mL at 60°C, 7 h; >7-log reduction PFU/mL (complete inactivation) at 60°C, 16 h | (Welch et al. 2006) |
| Birnaviridae | Infectious pancreatic necrosis virus | No | Media                | 37.5°C-60°C, 0-20 h | PRA    |                                                                           | (Gosting and Gould 1981) |
| Caliciviridae| Canine calicivirus           | No       | Media                | 4°C-100°C, (wks/sec) | TCID₅₀  | 3-log TCID₅₀/mL reduction at 71°C, 1 min                               | (Duizer et al. 2004) |
| Caliciviridae| Feline calicivirus           | No       | Media                | 37°C-60°C, 180 min | PRA    | >4-log PFU/mL reduction (complete inactivation) at 60°C, 30 min        | (Gibson and Schwab 2011) |
| Caliciviridae| Feline calicivirus           | No       | Media                | 4°C-100°C, (wks/sec) | TCID₅₀  | 3-log TCID₅₀/mL reduction at 71°C, 1 min                               | (Duizer et al. 2004) |
| Caliciviridae| Feline calicivirus           | No       | Buffered medium      | 50°C-72°C, 0-60 min | PRA    | >5-log PFU/mL reduction at 60°C, 5 min                                 | (Bozkurt et al. 2014a) |
| Caliciviridae| Feline calicivirus           | No       | Homogenized blue mussel | 50-72, 0-6 min  | PRA    | 4.9-log PFU/mL reduction at 60°C, 1 min; >7-log reduction (complete inactivation) at 65°C, 30 sec | (Bozkurt et al. 2014b) |
| Caliciviridae| Feline calicivirus           | No       | Turkey deli meat     | (1) 50°C, 0-6 min; (2) 56°C/60°C, 0-3 min; (3) 65°C/72°C, 0-90 sec | PRA    | >6-log PFU/g reduction (complete inactivation) at 65°C or 72°C, <30 sec | (Bozkurt et al. 2015a) |
| Caliciviridae| Human norovirus              | No       | Mussels              | 37°C-60°C, 180 min | qRT-PCR | 3-log reduction (complete inactivation) at 80°C, 30 min; 2.8-log reduction at 90°C, 15 sec | (Hewitt and Greening 2006) |
| Caliciviridae| Human norovirus              | No       | Media                | 60°C-90°C, 2 min  | qRT-PCR | >7.5-log reduction IU/mL (complete inactivation) at 100°C, 3 min       | (Li et al. 2017) |
| Caliciviridae| Murine norovirus             | No       | Media                | 100°C, 3 min     | qRT-PCR | >1.86-log PFU reduction at 65°C, 30 sec; 2.81-log PFU reduction at 75°C, 15 sec | (Duizer et al. 2015) |
| Caliciviridae| Murine norovirus             | No       | Raspberry puree      | (1) 65°C, 30 sec; (2) 75°C, 15 sec | PRA    | 1.86-log PFU reduction at 65°C, 30 sec; 2.81-log PFU reduction at 75°C, 15 sec | (Baert et al. 2008) |
| Caliciviridae| Murine norovirus             | No       | Bovine milk/water    | 63°C-72°C, 0-10 min | PRA    | >5.5-log PFU/mL reduction (complete inactivation) at 63°C, 10 min (milk) | (Hewitt et al. 2009) |
| Caliciviridae| Murine norovirus             | No       | Media                | 37°C-60°C, 180 min | PRA    | >5-log PFU/mL reduction (complete inactivation) at 60°C, 60 min        | (Gibson and Schwab 2011) |
| Caliciviridae| Murine norovirus             | No       | Soft-shell clams     | 85°C, 90°C, 90-300 sec | PRA    | >5-log PFU/mL reduction (complete inactivation) at 90°C, 180 sec      | (Sow et al. 2011) |
| Caliciviridae| Murine norovirus             | No       | Cell culture lysate  | 70°C, 85°C, 100°C, 0.5-10 min | PRA    | >4-log PFU/mL reduction at 70°C, 10 min; 7-log PFU/mL reduction (complete inactivation) at 85°C, 1 min | (Park et al. 2014a) |
| Caliciviridae| Murine norovirus             | No       | Buffered medium      | 50°C-72°C, 0-60 min | PRA    | >5-log PFU/mL reduction at 60°C, 5 min                                 | (Bozkurt et al. 2014a) |
| Caliciviridae| Murine norovirus             | No       | Homogenized          | 50-72, 0-6 min    | PRA    | 2.2-log PFU/mL reduction at 60°C, 1 min; >6-log reduction (complete inactivation) at 60°C, 45 min | (Bozkurt et al. 2014b) |
| Family          | Virus Type         | Presence | Sample Type       | Temperature Range | Time (min) | TCID<sub>50</sub> Reduction | Complete Inactivation | Source |
|-----------------|--------------------|----------|-------------------|-------------------|------------|------------------------------|----------------------|--------|
| Caliciviridae   | Murine norovirus   | No       | Media             | 60°C-85°C, 0-30 min | TCID<sub>50</sub> | >3-log TCID<sub>50</sub>/mL reduction at 72°C, 20 sec | >7-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 85°C, 10 min | (Park et al. 2014b) |
| Caliciviridae   | Murine norovirus   | No       | Turkey deli meat  | (1) 50°C, 0-6 min; (2) 56°C, 60°C, 0-3 min; (3) 65°C, 72°C, 0-90 sec | PRA | >5-log PFU/g reduction (complete inactivation) | at 65°C or 72°C, <30 sec | (Bozkurt et al. 2015a) |
| Caliciviridae   | Murine norovirus   | No       | Media             | (1) 62°C, 30 sec; (2) 72°C, 80 sec; (3) 80°C, 12 sec | PRA | >6-log PFU/mL reduction (complete inactivation) at 62°C, 24 min |                      | (Araud et al. 2016) |
| Caliciviridae   | Murine norovirus   | No       | Oyster homogenate | 49°C-67°C, 0-5 min | PRA | >3-log PFU/mL reduction at 63°C, 2 min; >5-log PFU/mL reduction (complete inactivation) at 67°C, 1 min |                      | (Shao et al. 2018) |
| Caliciviridae   | Tulane virus       | No       | Media             | (1) 62°C, 30 sec; (2) 72°C, 80 sec; (3) 80°C, 12 sec | PRA | >6-log PFU/mL reduction (complete inactivation) at 62°C, 30 min |                      | (Araud et al. 2016) |
| Caliciviridae   | Tulane virus       | No       | Media             | 60°C-90°C, 2 min | PRA | >4-log PFU/mL reduction at 60°C, 2 min; 5-log PFU/mL reduction at 80°C, 2 min |                      | (Li et al. 2017) |
| Caliciviridae   | Tulane virus       | No       | Oyster homogenate | 49°C-67°C, 0-5 min | PRA | >2-log PFU/mL reduction at 63°C, 30 sec; >3-log reduction (complete inactivation) at 63°C, 1 min |                      | (Shao et al. 2018) |
| Caliciviridae   | Tulane virus       | No       | Media             | 37°C-72°C, 1-30 min | TCID<sub>50</sub> | >5-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 63°C, 5 min |                      | (Tian et al. 2013) |
| Caliciviridae   | Tulane virus       | No       | Media             | 52°C, 54°C, 56°C, 10 min | PRA | >6-log PFU/mL reduction (complete inactivation) at 56°C, 10 min |                      | (Ailavadi et al. 2019) |
| Circoviridae    | Porcine circovirus | 2 No     | Factor VIII       | 65°C-75°C, 30 min | TCID<sub>50</sub> | 0.25-log TCID<sub>50</sub>/mL reduction at 65°C, 30 min; 1.92-log TCID<sub>50</sub>/mL reduction at 75°C, 30 min; >6.5-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 56°C, 60 min |                      | (Welch et al. 2006) |
| Coronaviridae   | Canine coronavirus | Yes      | Blood plasma      | 65°C, 0-10 h | TCID<sub>50</sub> | >7-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 65°C, 15 min |                      | (Leclercq et al. 2014) |
| Coronaviridae   | MERS-CoV           | Yes      | Blood plasma      | 65°C, 0-120 min | TCID<sub>50</sub> | >6-log TCID<sub>50</sub>/mL reduction (complete inactivation) |                      | (Duan et al. 2003) |
| Coronaviridae   | MERS-CoV           | Yes      | Media             | 37°C-75°C, 0-120 min | TCID<sub>50</sub> | >7-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 65°C, 15 min |                      | (Duan et al. 2003) |
| Coronaviridae   | Mouse hepatitis virus | Yes   | Blood plasma      | 65°C, 0-120 min | TCID<sub>50</sub> | >5-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 65°C, 15 min |                      | (Kariwa et al. 2006) |
| Coronaviridae   | SARS-CoV           | Yes      | Media             | 56°C, 0-90 min | TCID<sub>50</sub> | >7-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 56°C, 30 min; >7-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 56°C, 60 min |                      | (Yunoki et al. 2004) |
| Coronaviridae   | SARS-CoV           | Yes      | Plasma product    | 60°C, 120 min | TCID<sub>50</sub> | >6-log TCID<sub>50</sub>/mL reduction at 60°C, 30-60 min; >6-log TCID<sub>50</sub> reduction (complete inactivation) at 60°C, 60 min |                      | (Darnell et al. 2004) |
| Coronaviridae   | SARS-CoV           | Yes      | Media             | 56°C-75°C, 0-90 min | TCID<sub>50</sub> | >6-log TCID<sub>50</sub>/mL reduction at 56°C, 20 min; >4-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 65°C, 10 min |                      | (Darnell et al. 2004) |
| Coronaviridae   | SARS-CoV           | Yes      | HSA               | 56°C, 65°C, 0-120 min | TCID<sub>50</sub> | >5-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 56°C, 20 min |                      | (Darnell and Taylor 2006) |
| Family          | Virus Type                        | Presence | Media/Albumin | Temperature   | Time   | TCID<sub>50</sub> or PRA Reduction | Reference                                      |
|-----------------|-----------------------------------|----------|---------------|---------------|-------|------------------------------------|------------------------------------------------|
| Coronaviridae   | SARS-CoV-2                         | Yes      | Media         | 56°C, 70°C, 1-30 min | TCID<sub>50</sub> | >6-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 56°C, 30 min; >6-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 70°C, 5 min | (Chin et al. 2020) |
| Coronaviridae   | Transmissible gastroenteritis virus | Yes      | Media         | 31°C-55°C, 0-80 h | PRA   | >5-log PFU/mL reduction at 55°C, 60 min | (Laude 1981)                                    |
| Flaviviridae    | Alkhurma hemorrhagic fever virus   | Yes      | Media         | 45°C-60°C, 0-60 min | TCID<sub>50</sub> | >7-log TCID<sub>50</sub> reduction (complete inactivation) at 60°C, 3 min | (Madani et al. 2014)                           |
| Flaviviridae    | Bovine viral diarrhea virus       | Yes      | FBS           | 56°C, 15-45 min | TCID<sub>50</sub> | Complete inactivation (reduction factor >4.88) at 56°C, 15 min | (Danner et al. 1999)                           |
| Flaviviridae    | Bovine viral diarrhea virus       | Yes      | Bovine serum albumin/transferrin solution | Diasprin crosslinked hemoglobin | 74°C, 90 min | PRA | >6.7-log PFU reduction (complete inactivation) at 74°C, 90 min | (Azari et al. 1998) |
| Flaviviridae    | Bovine viral diarrhea virus       | Yes      | Immunoglobulin preparation | Media | 60°C, 10 h | TCID<sub>50</sub> | >6-log TCID<sub>50</sub>/mL reduction at 60°C, 10 h | (Aghaie et al. 2008)                           |
| Flaviviridae    | Bovine viral diarrhea virus       | Yes      | Media         | 40°C-95°C, 1-2 h | TCID<sub>50</sub> | >4-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 95°C, 2 h | (Sauerbrei and Wutzler 2009)                   |
| Flaviviridae    | Classical swine fever virus       | Yes      | Media         | 55°C-70°C, 0-15 min | TCID<sub>50</sub> | >7-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 65°C, 2 min | (Turner et al. 2000)                           |
| Flaviviridae    | Hepatitis C virus                 | Yes      | Media/Human serum | 56°C-65°C, 0-40 min | IF (Focus-forming unit) | >4-log FFU/mL reduction (complete inactivation) at 65°C, 4 min | (Song et al. 2010)                              |
| Flaviviridae    | Tick-borne encephalitis virus     | Yes      | Antithrombin III solution | Serum albumin | 60°C, 0-10 h | PRA | >7-log PFU/mL reduction (complete inactivation) at 60°C, 180 min | (Barrett et al. 1996)                           |
| Flaviviridae    | Zika virus                        | Yes      | Serum albumin | 57°C-58°C, 0-600 min | TCID<sub>50</sub> | >4-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 57°C, ramp-up time to 57°C | (Farcet and Kreil 2017)                        |
| Flaviviridae    | Zika virus                        | Yes      | Media         | 56°C, 10 min | TCID<sub>50</sub> | >5-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 56°C, 5 min | (Blümel et al. 2017)                           |
| Hepadnaviridae  | Duck Hepatitis B virus            | Yes      | Hemoglobin solutions | Media | 60°C, 1 h | Bioassay | >6-log DIU/mL reduction (complete inactivation) at 60°C, 1h | (Farmer et al. 1992)                           |
| Hepadnaviridae  | Duck Hepatitis B virus            | Yes      | HSA           | 60°C, 10 h | RIFA | 6.5-log RIFU/mL reduction (complete inactivation, no IF+ cells) at 60°C, 60 min | (Adcock et al. 1998)                           |
| Hepadnaviridae  | Hepatitis B virus                 | Yes      | Serum albumin | 37°C, 56°C, 30-600 min | FQ-PCR | >1-log copies/mL reduction at 56°C, 60 min; >2-log copies/mL reduction at 56°C, 600 min Complete inactivation (no Hepatitis E + cells) | (Song et al. 2011)                              |
| Hepeviridae     | Hepatitis E virus                 | No       | Fecal suspension | 45-70°C, 1 h | IF | 1.2-log reduction at 62°C, 5 min; 2.6-log reduction at 71°C 10 min | (Emerson et al. 2005)                           |
| Hepeviridae     | Hepatitis E virus                 | No       | Homogenized liver | 62°C-71°C, 5-120 min | Bioassay | >3-log IU/mL reduction (complete inactivation) at 65°C, 5 min (media); >3-log reduction IU/mL (complete inactivation) at 70°C, 5 min (minced pork) | (Barnaud et al. 2012)                          |
| Hepeviridae     | Hepatitis E virus                 | No       | Media/minced pork | (1) 62°C, 1-30 min (2) 65°C, 70°C, 1-5 min | RT-PCR | 4-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 60°C, ≤2h | (Imagawa et al. 2018)                          |
| Herpesviridae   | Bovine herpes virus               | Yes      | Bovine serum albumin/transferrin solution | Media | 60°C-61°C, 10 h | TCID<sub>50</sub> | >4-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 60°C, 30 min | (Plavsic 2000)                                  |
| Herpesviridae   | Bovine herpes virus               | Yes      | FBS           | 56°C, 15-45 min | TCID<sub>50</sub> | Complete inactivation (reduction factor >27.24) at 56°C, 30 min | (Danner et al. 1999)                           |
| Virus Family   | Virus Type                  | Pathogen   | Medium/Repository          | Temperature | Incubation Time | TCID<sub>50</sub> | Reduction at 60°C | Source                          |
|---------------|----------------------------|------------|----------------------------|-------------|-----------------|-------------------|-------------------|--------------------------------|
| Herpesviridae | Bovine herpes virus        | Yes        | Immunoglobulin preparation | 60°C, 10 h  | TCID<sub>50</sub> | 6-log TCID<sub>50</sub>/mL reduction at 60°C, 120 min; 5-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 60°C, 240 min | (Hosseini et al. 2014) |
| Herpesviridae | Cytomegalovirus            | Yes        | Media                      | 50°C, 0-60 min | PRA             | >4-log PFU/mL reduction at 50°C, 30 min; > 6-log PFU/mL reduction at 50°C, 40 min | (Plummer and Lewis 1965) |
| Herpesviridae | Cytomegalovirus            | Yes        | Blood plasma                | 65°C, 0-10 h | TCID<sub>50</sub> | Complete inactivation at 65°C, 15 min | (Lelie et al. 1987) |
| Herpesviridae | Cytomegalovirus            | Yes        | Hemoglobin solutions        | 60°C, 1 h    | PRA             | >6-log PFU/mL reduction (complete inactivation) at 60°C, 30 min | (Farmer et al. 1992) |
| Herpesviridae | Cytomegalovirus            | Yes        | Infant formula              | 62.5°C, 30 min | PRA             | >3-log PFU/mL reduction (complete inactivation) at 65°C, 30 min | (Mikawa et al. 2019) |
| Herpesviridae | Duck plague virus          | Yes        | Media                      | 42°C-96°C, 2 h | TCID<sub>50</sub> | >6-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 56°C, 2 h | (Makhija and Kumar 2017) |
| Herpesviridae | Herpes simplex virus       | Yes        | Media                      | 50°C, 0-60 min | PRA             | >10-log PFU/mL reduction (complete inactivation) at 50°C, 20 min | (Plummer and Lewis 1965) |
| Herpesviridae | Herpes simplex virus       | Yes        | Bovine milk                 | 40°C-85°C, 0-30 min | PRA             | 4-log PFU/mL reduction at 60°C, 2 sec | (Sullivan et al. 1971) |
| Herpesviridae | Pseudorabies virus         | Yes        | Immunoglobulin preparation | 60°C, 10 h   | TCID<sub>50</sub> | >5-log TCID<sub>50</sub>/mL reduction at 60°C, 10 h | (Aghaie et al. 2008) |
| Herpesviridae | Pseudorabies virus         | Yes        | Antithrombin III solution   | 60°C, 0-10 h | TCID<sub>50</sub> | >7-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 60°C, 10 min | (Koch et al. 2011) |
| Orthomyxoviridae | High pathogenicity avian influenza | Yes        | Fat-free egg products     | 53°C-63°C, 0-40 min | TCID<sub>50</sub> | >5-log TCID<sub>50</sub>/mL reduction at 59°C, 2 min | (Chmielewski et al. 2011) |
| Orthomyxoviridae | Low pathogenicity avian influenza | Yes        | Fat-free egg products     | 53°C-63°C, 0-40 min | TCID<sub>50</sub> | >7-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 62°C, 10 min | (Chmielewski et al. 2011) |
| Orthomyxoviridae | Influenza virus            | Yes        | Allantoic fluid            | 46-54°C, 15 min | EID<sub>50</sub> | Infectivity reduced significantly at 54°C, 15 min | (Chu 1948) |
| Orthomyxoviridae | Influenza virus            | Yes        | Allantoic fluid            | 56°C, 0-8 h  | EID<sub>50</sub> | >90% reduction in infectivity at 56°C,22 min | (De Flora and Badolati 1973) |
| Orthomyxoviridae | Influenza virus            | Yes        | Blood plasma               | 65°C, 0-10 h | TCID<sub>50</sub> | >3-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 65°C, 15 min | (Lelie et al. 1987) |
| Paramyxoviridae | Virulent Newcastle disease virus | Yes        | Fat-free egg products     | 53°C-63°C, 0-40 min | TCID<sub>50</sub> | >5-log TCID<sub>50</sub>/mL reduction at 59°C, 10 min | (Chmielewski et al. 2011) |
| Paramyxoviridae | Low-virulent Newcastle disease virus | Yes        | Fat-free egg products     | 53°C-63°C, 0-40 min | TCID<sub>50</sub> | >5-log TCID<sub>50</sub>/mL reduction at 59°C, 3 min | (Chmielewski et al. 2011) |
| Paramyxoviridae | Measles virus              | Yes        | Media                      | 37°C-56°C, 0-120 min | PRA             | Survival ratio 1/1000 at 52°C or 56°C <15 min | (Arita and Matumoto 1968) |
| Paramyxoviridae | Newcastle disease virus    | Yes        | Allantoic fluid            | 54-58°C, 15 min | EID<sub>50</sub> | Infectivity reduced significantly at 58°C, 15 min | (Chu 1948) |
| Paramyxoviridae | Newcastle disease virus    | Yes        | Chicken homogenate         | 60°C-80°C, 0-10 min | EID<sub>50</sub> | 4-log EID<sub>50</sub>/reduction at 60°C, 10 min; >6-log EID<sub>50</sub>/reduction (complete inactivation) at 80°C, 10 sec | (Alexander and Manvell 2004) |
| Paramyxoviridae | Paramyxovirus type 3 virus | Yes        | FBS                        | 56°C, 15-45 min | TCID<sub>50</sub> | Complete inactivation (reduction factor >35.58) at 56°C for 15 min | (Danner et al. 1999) |
| Parvoviridae | Canine parovirus          | No         | Blood plasma               | (1) 103°C, 90 sec; (2) 65°C, 0-10 h | TCID<sub>50</sub> | >2-log TCID<sub>50</sub>/mL reduction 65°C, 40 min; 5.5-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 103°C, 90 sec | (Lelie et al. 1987) |
| Family         | Species                          | Matrix Type | Temp/Time | TCID<sub>50</sub> | Inactivation Details                                                                 |
|---------------|----------------------------------|-------------|-----------|-------------------|--------------------------------------------------------------------------------------|
| Parvoviridae  | Porcine parovirus                | No          | FBS       | 56°C, 15-45 min   | TCID<sub>50</sub> Incomplete inactivation (reduction factor 1.09) at 56°C for 45 min (Danner et al. 1999) |
| Parvoviridae  | Porcine parovirus                | No          | HSA       | 30°C, 1-60 min    | TCID<sub>50</sub> <1-log TCID<sub>50</sub>/mL reduction at 60°C, 60 min (Blumel et al. 2002) |
| Parvoviridae  | Porcine parovirus                | No          | Diaspirin crosslinked hemoglobin Media | 40°C-95°C, 1-2 h | TCID<sub>50</sub> 0.9-log TCID<sub>50</sub>/mL reduction at 95°C, 2 h (Sauerbrei and Wutzler 2009) |
| Parvoviridae  | Bovine parovirus                 | No          | Antithrombin III solution | 60°C, 0-10 h | TCID<sub>50</sub> >3-log TCID<sub>50</sub>/mL reduction at 60°C, 10 h (Barrett et al. 1996) |
| Picornaviridae| Bovine enterovirus               | No          | Bovine serum albumin/transferin solution | 60°C-61°C, 10 h | TCID<sub>50</sub> >4-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 60°C, ≤2 h (Plavsic 2000) |
| Picornaviridae| Encephalomyocarditis virus       | No          | Blood plasma | 65°C, 0-10 h | TCID<sub>50</sub> >9-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 65°C, 15 min (Leile et al. 1987) |
| Picornaviridae| Foot and mouth disease virus     | No          | Human plasma | 60°C-90°C, 0.25 sec | TCID<sub>50</sub> >5.8-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 72°C, 0.25 sec (Charm et al. 1992) |
| Picornaviridae| Foot and mouth disease virus     | No          | Media     | 55°C-61°C, 0-8 h  | TCID<sub>50</sub> >3-log TCID<sub>50</sub>/mL reduction at 55°C, 8 min; >5-log TCID<sub>50</sub>/mL reduction at 61°C, 20 sec (Bachrach et al. 1957) |
| Picornaviridae| Foot and mouth disease virus     | No          | Bovine milk | 56°C-85°C, 0-60 min | TCID<sub>50</sub> 5-log PFU/mL reduction at 63°C, 1 min (Sellers 1969) |
| Picornaviridae| Foot and mouth disease virus     | No          | Bovine milk | 72°C-80°C (15-17 sec) | PFU/mL 3.7-5.5-log PFU/mL reduction at 72°C, 15-17 sec; 4.7-6.0-log PFU/mL reduction at 80°C, 15-17 sec (Hyde et al. 1975) |
| Picornaviridae| Foot and mouth disease virus     | No          | Media     | 55°C-70°C, 0-15 min | TCID<sub>50</sub> >7-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 60°C, 10 min (Turner et al. 2000) |
| Picornaviridae| Foot and mouth disease virus     | No          | Immunoglobulin preparation | 60°C, 10 h | TCID<sub>50</sub> 5-log reduction TCID<sub>50</sub>/mL at 60°C, 120 min; 7-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 60°C, 240 min (Hosseini et al. 2014) |
| Picornaviridae| Foot and mouth disease virus     | No          | Bovine milk | 73°C/80°C; 18-36 sec | TCID<sub>50</sub> >2-3-log PFU/mL reduction (complete inactivation) at 73°C, 18 sec (Tomassula et al. 2007) |
| Picornaviridae| Foot and mouth disease virus     | No          | Bovine milk | 72°C, 0-5 min | TCID<sub>50</sub> 5-6-log PFU/mL reduction (complete inactivation) at 72°C, 2 min in whole and skim milk (Blackwell and Hyde 1976) |
| Picornaviridae| Hepatitis A virus                | No          | Bovine milk/PBS | (1) 62.8, 30 min; (2) 71.6, 15 sec | TCID<sub>50</sub> >3-log TCID<sub>50</sub>/mL reduction at 62.8°C, 30 min (milk); 5-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 62.8°C, 30 min (PBS) (Parry and Mortimer 1988) |
| Picornaviridae| Hepatitis A virus                | No          | Media     | 37°C-70°C, 5-60 min | TCID<sub>50</sub> 1-log TCID<sub>50</sub>/mL reduction at 50°C, 60 min; 4-log TCID<sub>50</sub>/mL reduction 60°C, 60 min; >6-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 70°C, 30 min (Flehming et al. 1985) |
| Picornaviridae| Hepatitis A virus                | No          | 0.1M NaCl or 2M MgCl<sub>2</sub> Media | 20°C, 60°C, 10 min | TCID<sub>50</sub> 3.3-log PFU/mL reduction at 60°C,10 min (Anderson 1987) |
| Picornaviridae| Hepatitis A virus                | No          | Antithrombin III solution | 60°C, 0-10 h | TCID<sub>50</sub> >3.6-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 60°C, 30 min (Murphy et al. 1993) |
| Picornaviridae| Hepatitis A virus                | No          | Media/Mussel homogenate | (1) 60°C, 10-30 min; (2) 80°C, 3-15 min; (3) 100°C, 1-8 min | TCID<sub>50</sub> >5-log TCID<sub>50</sub>/mL reduction at 60°C, 10 min (media); >5-log TCID<sub>50</sub>/mL at 60°C,15 min (homogenate) (Croci et al. 1999) |
| Picornaviridae| Hepatitis A virus                | No          | Bovine milk | (1) 0-16 min, 65-70°C; | PRA 5-log PFU/mL reduction at 65°C, 41-46 min (all (Badowid et al. 2000) |
| Picornaviridae | Hepatitis A virus | No | Products | IF | TCID<sub>50</sub> | 1-log PFU/mL reduction | 3-log PFU/mL reduction | Complete inactivation |
|---------------|------------------|----|----------|----|-----------------|------------------------|------------------------|----------------------|
| Picornaviridae | Hepatitis A virus | No | Fecal suspension | 45-70°C, 1 h |  |  |  | Emerson et al. (2005) |
| Picornaviridae | Hepatitis A virus | No | Mussels | (steam, boil), 37 sec, 180 sec | TCID<sub>50</sub> | 2-log TCID<sub>50</sub>/mL reduction | 5-log PFU/mL reduction | Hewitt and Greening (2006) |
| Picornaviridae | Hepatitis A virus | No | Bovine milk/water | 63°C-72°C, 0-10 min | PRA | 3.5-log PFU/mL reduction | 5-log PFU/mL reduction | Hewitt et al. (2009) |
| Picornaviridae | Hepatitis A virus | No | HSA | 60°C, 0-10 h | IF | 1-log FFU reduction | 3-log PFU/mL reduction | Shimasaki et al. (2009) |
| Picornaviridae | Hepatitis A virus | No | Media | 37°C-7°C, 180 min | PRA | 4.5-log PFU/mL reduction | 6-log PFU/mL reduction | Gibson and Schwab (2011) |
| Picornaviridae | Hepatitis A virus | No | Green onions | 45-65°C, 20 h dehydration | PRA | 2-log PFU/mL reduction | 5-log PFU/mL reduction | Laird et al. (2011) |
| Picornaviridae | Hepatitis A virus | No | Soft-shell clams | 85°C, 90°C, 90-300 sec | PRA | 3-log PFU/mL reduction | 5-log PFU/mL reduction | Sow et al. (2011) |
| Picornaviridae | Hepatitis A virus | No | Mussels | Steam: 50°C-100°C | PRA | 3-log PFU/mL reduction | 6-log PFU/mL reduction | (Harlow et al. 2011) |
| Picornaviridae | Hepatitis A virus | No | HSA | 58°C, 600 min | TCID<sub>50</sub> | 3.1-5.2-log TCID<sub>50</sub>/mL reduction at 58°C for 600 min (4.5-25% serum albumin) | (Farce et al. 2012) |
| Picornaviridae | Hepatitis A virus | No | Manila clams | 60°C, 10 min | TCID<sub>50</sub> | 2-log reduction at 60°C, 10 min | (Cappellozza et al. 2011) |
| Picornaviridae | Hepatitis A virus | No | Buffered medium | 50°C-72°C, 0-60 min | PRA | 3-log PFU/mL reduction at 60°C, 10 min | (Bozkurt et al. 2014a) |
| Picornaviridae | Hepatitis A virus | No | Spinach | 50°C-72°C, 0-6 min | PRA | 2-log PFU/mL reduction | 5-log PFU/mL reduction | (Bozkurt et al. 2015c) |
| Picornaviridae | Hepatitis A virus | No | Homogenized clam meat | 50°C-72°C, 0-6 min | PRA | 1-log PFU/mL reduction | 5-log PFU/mL reduction | (Bozkurt et al. 2015b) |
| Picornaviridae | Hepatitis A virus | No | Turkey deli meat | (1) 50°C, 0-6 min; (2) 56°C-60°C, 0-3 min; (3) 63°C-72°C, 0-90 sec; (1) 62°C, 30 sec; (2) 72°C, 80 sec; (3) 80°C, 12 sec | PRA | 1-log PFU/mL reduction | 7-log PFU/mL reduction | (Bozkurt et al. 2015a) |
| Picornaviridae | Hepatitis A virus | No | Media | 60°C, 10 h | TCID<sub>50</sub> | 8-log reduction TCID<sub>50</sub>/mL at 60°C, 10 h | (Aghaie et al. 2008) |
| Picornaviridae | Poliovirus | No | 0.1M NaCl or 2M MgCl<sub>2</sub> | 20°C, 60°C, 10 min | PRA | 4-log PFU/mL reduction (complete inactivation) | (Anderson 1987) |
| Picornaviridae | Poliovirus | No | Hemoglobin solutions | 60°C, 0-10 h | PRA | 6-log PFU/mL reduction (complete inactivation) | (Estep et al. 1988) |
| Picornaviridae | Poliovirus | No | Immunoglobulin preparation | 60°C, 10 h | TCID<sub>50</sub> | 8-log reduction TCID<sub>50</sub>/mL at 60°C, 10 h | (Aghaie et al. 2008) |
| Picornaviridae | Poliovirus | No | Media | 60°C, 10 h | TCID<sub>50</sub> | 5-log TCID<sub>50</sub>/mL reduction (complete inactivation) | (Murphy et al. 1993) |
| Picornaviridae | Poliovirus | No | Bovine milk/water | (1) 62°C, 30 min; (2) 72°C, 15-30 sec | PRA | 5-log PFU/mL reduction at 62°C, 30 min | (Strazynski et al. 2002) |
| Picornaviridae | Poliovirus | No | Media | 40°C-95°C, 1-2 h | TCID<sub>50</sub> | 4.8-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 85°C, 1 h | (Sauerbrei and Wutzler 2009) |
| Polyomaviridae | Simian virus 40 | No | Blood plasma | (1) 103°C, 90 sec; (2) | TCID<sub>50</sub> | 4-log TCID<sub>50</sub>/mL reduction at 103°C, 90 sec | (Lelie et al. 1987) |
| Family                  | Virus Name                      | Species | Matrix | TEMPERATURE | METHOD | TCID<sub>50</sub> REDUCTION | REFERENCE |
|------------------------|---------------------------------|---------|--------|-------------|--------|-------------------------------|-----------|
| Polyomaviridae         | Polyomavirus SV40               | No      | Media  | 65°C, 0-10 h | TCID<sub>50</sub> | >5.1-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 95°C, 2 h | (Sauerbrei and Wutzler 2009) |
| Poxviridae             | Vaccinia virus                  | Yes     | Blood plasma | 65°C, 0-10 h | TCID<sub>50</sub> | >5.8-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 65°C, 15 min | (Lelie et al. 1987) |
| Poxviridae             | Vaccinia virus                  | Yes     | Media   | 40°C-95°C, 1-2 h | TCID<sub>50</sub> | >4.3-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 95°C, 2 h | (Sauerbrei and Wutzler 2009) |
| Reoviridae             | Reovirus                        | No      | Bovine milk | 40°C-85°C, 0-30 min | PRA | >5-log PFU/mL reduction at 60°C, 12 sec | (Sullivan et al. 1971) |
| Reoviridae             | Reovirus                        | No      | Immunoglobulin preparation | 60°C, 10 h | TCID<sub>50</sub> | >7-log TCID<sub>50</sub>/mL reduction at 60°C, 10 h | (Aghaie et al. 2008) |
| Reoviridae             | Reovirus                        | No      | FBS     | 56°C, 15-45 min | TCID<sub>50</sub> | Complete inactivation (reduction factor >5.50) at 56°C for 30 min | (Danner et al. 1999) |
| Reoviridae             | Avian Reovirus                  | No      | Bovine serum albumin/transfer preparation | 60°C-61°C, 10 h | TCID<sub>50</sub> | >4-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 60°C, ≤2h | (Plavsic 2000) |
| Reoviridae             | Human Rotavirus                 | No      | Media   | (1) 62°C, 30 sec; (2) 72°C, 80 sec; (3) 80°C, 12 sec; (4) 37°C, 47°C, 62.8°C (milk only), 30 min | PRA | >2-log PFU/mL reduction at 62°C, 30 min; >6-log PFU/mL reduction (complete inactivation) at 72°C, 60 sec | (Araud et al. 2016) |
| Retroviridae           | Bovine immunodeficiency virus   | Yes     | Media/Bovine milk | 56°C-73°C, 0.5-1 min | TCID<sub>50</sub> | Complete inactivation at temperatures >60°C | (Baumgartner et al. 1976) |
| Retroviridae           | Bovine leukemia virus           | Yes     | Media   | (1) 63°C, 30 min; (2) 72-73°C, 15-20 sec | TCID<sub>50</sub> | Complete inactivation at 63°C, 30 min; Complete inactivation at 72°C-73°C, 15-20 sec | (Chung et al. 1986) |
| Retroviridae           | Bovine leukemia virus           | Yes     | Blood plasma | 65°C, 0-10 h | TCID<sub>50</sub> | >4-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 65°C, 15 min | (Lelie et al. 1987) |
| Retroviridae           | Bovine leukemia virus           | Yes     | Hemoglobin solutions | 60°C, 0-10 h | PRA | Complete inactivation at 60°C, 7 min | (Estep et al. 1988) |
| Retroviridae           | Bovine leukemia virus           | Yes     | Media   | 60°C, 10-240 min | TCID<sub>50</sub> | 5.5-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 60°C, 10 min | (Gregersen et al. 1989) |
| Retroviridae           | Bovine leukemia virus           | Yes     | Hemoglobin solutions | 60°C, 1 h | ELISA | >4-log IU/mL reduction (complete inactivation) at 60°C, 30 min | (Farmer et al. 1992) |
| Retroviridae           | Bovine leukemia virus           | Yes     | Antithrombin III solution | 60°C, 0-10 h | TCID<sub>50</sub> | >6-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 60°C, 20 min | (Barrett et al. 1996) |
| Retroviridae           | Bovine leukemia virus           | Yes     | Media   | 60°C, 10-240 min | TCID<sub>50</sub> | 5-log TCID<sub>50</sub>/mL reduction (complete inactivation), 60°C, 10 min | (Gregersen et al. 1989) |
| Retroviridae           | Human T lymphotrophic virus III | Yes     | Human plasma | 60°C-90°C, 0.25 sec | TCID<sub>50</sub> | >4.4-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 77°C-80°C, 0.25 sec | (Charm et al. 1992) |
| Retroviridae           | Human T lymphotrophic virus III | Yes     | Human serum | 56°C, 0-30 min | TCID<sub>50</sub> | >5-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 56°C, 10 min | (Martin et al. 1985) |
| Retroviridae           | Human T lymphotrophic virus III | Yes     | Serum   | 56°C, 1-60 min | IF | 88% reduction in infectivity at 56°C, 2.5 min; complete inactivation at 56°C after 30 min | (Harada et al. 1985) |
| Retroviridae           | Human T lymphotrophic virus III | Yes     | Media/serum/ factor VIII | 37°C-60°C, 0-120 min | TCID<sub>50</sub> | >6-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 60°C, 2 min in all liquid matrices 63% inactivation at 48°C at 30 min; <100% inactivation at 56°C at 20 min | (McDougal et al. 1985) |
| Retroviridae           | Lymphadenopathy- associated virus | Yes     | Media   | 37°C-56°C, 0-30 min | RT | >5-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 65°C, 15 min | (Spire et al. 1985) |
| Retroviridae           | Murine leukemia virus           | Yes     | Blood plasma | 65°C, 0-10 h | TCID<sub>50</sub> | >5-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 65°C, 15 min | (Lelie et al. 1987) |
| Retroviridae           | Rous sarcoma virus              | Yes     | Media   | 60°C, 10-240 min | TCID<sub>50</sub> | 4-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 60°C, 10 min | (Gregersen et al. 1989) |
### Table 3. Comparing the log reductions in detectable live viruses pasteurized in both a human milk and a non-human milk matrix

| Family          | Virus Name                                      | Matrix          | Media/Conditions          | Log Reduction | Inactivation Temperature | References |
|-----------------|------------------------------------------------|-----------------|---------------------------|---------------|--------------------------|------------|
| Rhabdoviridae   | Infectious hematopoietic necrosis virus         | Yes             | Media, 28°C-38°C, 0-400 min | PRA           | >7-log PFU/mL reduction at 38°C, 140 min | (Gosting and Gould 1981) |
| Rhabdoviridae   | Vesicular stomatitis virus                     | Yes             | Blood plasma, 65°C, 0-10 h | TCID<sub>50</sub> | >3-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 65°C, 15 min | (Lelie et al. 1987) |
| Rhabdoviridae   | Vesicular stomatitis virus                     | Yes             | Human plasma, 60°C-90°C, 0.25 sec | TCID<sub>50</sub> | >4.4-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 75°C, 0.25 sec | (Charm et al. 1992) |
| Rhabdoviridae   | Vesicular stomatitis virus                     | Yes             | Immunoglobulin preparation, 60°C, 10 h | TCID<sub>50</sub> | >6-log TCID<sub>50</sub>/mL reduction at 60°C, 10 h | (Aghaie et al. 2008) |
| Togaviridae     | Chikungunya virus                              | Yes             | Various, 56°C, 0-120 min | TCID<sub>50</sub> | 2.7-log TCID<sub>50</sub>/mL reduction at 56°C, 15 min; >5-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 56°C, 60 min | (Yue et al. 2019) |
| Togaviridae     | Chikungunya virus                              | Yes             | Media, 35°C-70°C, 1.5 min | TCID<sub>50</sub> | >4-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 60°C, 5 min | (Franz et al. 2018) |
| Togaviridae     | Mayaro virus                                   | Yes             | Various, 56°C, 0-120 min | TCID<sub>50</sub> | >2-log TCID<sub>50</sub>/mL reduction at 56°C, 30 min; >5-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 56°C, 90 min | (Yue et al. 2019) |
| Togaviridae     | Semliki forest virus                            | Yes             | PBS, 20°C-50°C, 0-60 min | PRA | Complete inactivation between 20°C and 50°C, 60 min | (Fleming 1971) |
| Togaviridae     | Sindbis virus                                  | Yes             | Blood plasma, 65°C, 0-10 h | TCID<sub>50</sub> | >10-log TCID<sub>50</sub>/mL reduction (complete inactivation) at 65°C, 15 min | (Lelie et al. 1987) |
| Togaviridae     | Sindbis virus                                  | Yes             | Hemoglobin solutions, 60°C, 0-10 h | PRA | >5-log PFU/mL reduction (complete inactivation) at 60°C, 30 min | (Estep et al. 1988) |
| Togaviridae     | Venezuelan equine encephalitis virus            | Yes             | Media, 58°C, 80°C, 1 h | PRA | 8-log PFU/mL reduction (complete inactivation) at 80°C, 1 h | (Patterson et al. 2018) |
| Tombusviridae   | Tobacco necrosis virus                          | No              | Water, 70°C-90°C, 0-180 min | ED | Complete inactivation at 80°C, 2-14 min | (Babos and Kassanis 1965) |

**Note.** Antigen, Ag: Endpoint dilution, ED; Egg infectious dose, EID; Fetal bovine serum, FBS; Fluorescence-focus unit, FFU; Fluorescence-quantitative polymerase chain reaction, FQ-PCR; Human serum albumin, HSA; Immunofluorescence, IF; Inactivation velocity, IV; Phosphate buffered saline, PBS; Plaque reduction assay, PRA; quantitative Reverse transcriptase(real time) polymerase chain reaction, qRT-PCR; Radio-immunofluorescence assay, RI; Reverse transcriptase, RT; Tissue culture infectious dose, TCID.
| Family          | Virus                                | Reduction* | Temperature † | Time ‡ | Reference                                      | Reduction* | Temperature † | Time ‡ | Reference                                      |
|-----------------|--------------------------------------|------------|---------------|--------|-----------------------------------------------|------------|---------------|--------|-----------------------------------------------|
| Caliciviridae   | Feline Calicivirus                   | >5         | 65            | 2      | (Topping et al. 2009)                         | 4.5 - >5   | 60            | 1-60   | (Gibson and Schwab 2011; Bozkurt et al. 2014b, 2014a) |
| Flaviviridae    | Bovine viral diarrhea virus          | ~4         | 72            | 0.1    | (Terpstra et al. 2007)                        | >6.5       | 60            | <120   | (Plavsic 2000; Aghaie et al. 2008)              |
| Flaviviridae    | Zika virus                           | >6         | 63            | 30     | (Hamilton Spence et al. 2017)                 | >4         | 56-58         | 5-10   | (Blümel et al. 2017; Farret and Kreil 2017)     |
| Herpesviridae   | Cytomegalovirus                      | >3/UD      | 56-63         | 8-30   | (Welsh et al. 1979; Friis and Andersen 1982; Goldblum et al. 1984) | >5/UD      | 50-65         | 15-30  | (Plummer and Lewis 1965; Lelie et al. 1987; Farmer et al. 1992; Mikawa et al. 2019) |
| Herpesviridae   | Herpes Simplex Virus                 | 4.2        | 63            | 30     | (Welsh et al. 1979)                           | >3 - >5    | 50-60         | <1-600 | (Plummer and Lewis 1965; Sullivan et al. 1971; Aghaie et al. 2008) |
| Picornaviridae  | Hepatitis A Virus                    | 2          | 72            | 0.3    | (Terpstra et al. 2007)                        | >3 - >5    | 60-63         | 10-60  | (Parry and Mortimer 1984; Flehmig et al. 1985; Anderson 1987; Murphy et al. 1993; Croci et al. 1999; Biddulph et al. 2000; Araud et al. 2016) |
| Parvoviridae    | Porcine Parvovirus                   | 0.5        | 72            | 0.3    | (Terpstra et al. 2007)                        | <1         | 56-60         | 15-60  | (Danner et al. 1999; Blumel et al. 2002)        |
| Retroviridae    | HIV-1                                | >5.5       | 62.5          | 30     | (Orloff et al. 1993)                          | >4         | 60-65         | 7-30   | (Lelie et al. 1987; Estep et al. 1988; Gregersten et al. 1989; Farmer et al. 1992) |
| Togaviridae     | Semliki forest virus                 | 3.2        | 63            | 30     | (Welsh et al. 1979)                           | UD         | 20-50         | 60     | (Fleming 1971)                                  |

**Note.** Undetectable, UD  
*Log-PFU or TCID₅₀/mL  
† Degree °C  
‡ Minutes
Figure Caption

Figure 1. PRISMA flow diagram describing the selection of studies for inclusion in the review.
Reports Identified through searching \((n=65131)\)

- MEDLINE (through 20/04/20): 23,441
- Embase (through 20/04/20): 34,479
- Web of Science (through 20/04/20): 7,200
- Manual Searches: 11

Reports excluded on basis of title and/or abstract \((n=64950)\)

- Duplicate records: 20,663
- Did not meet inclusion criteria: 44,287
  
  1) Review Paper  
  2) Clinical trial/Randomized controlled trial  
  3) Qualitative/Observational  
  4) Did not assess effect of heat on viral inactivation or load in a specific matrix  
  5) Non-Scientific Article

Reports reviewed in full \((n=181)\)

Reports excluded \((n=73)\)

- Did not meet inclusion criteria: 65
- Duplicate: 6
- Could not retrieve: 2

Studies included in review \((n=108)\)