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

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Abstract

Holder pasteurization (62.5ºC, 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 common 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, of 65,131 reports identified, 109 studies were included. Pasteurization of HM at a minimum temperature of 56ºC-60ºC is effective at reducing detectable live virus. In cell culture media or plasma, coronaviruses (e.g., SARS-CoV, SARS-CoV-2, MERS-CoV) are highly susceptible to heating at ≥56ºC. Although pasteurization parameters and matrices reported vary, all viruses studied, except parvoviruses, were susceptible to thermal killing. Future research important for the study of novel viruses should standardize pasteurization protocols and should test inactivation in human milk.

Novelty bullets

- In all matrices, including human milk, pasteurization at 62.5ºC was generally sufficient to reduce surviving viral load by several logs or to below the limit of detection.
- Holder pasteurization (62.5ºC, 30 min) of human 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

Breastfeeding is associated with numerous positive health and neurocognitive outcomes: these include lower infectious morbidity and mortality, higher intelligence and protection against the development of chronic disease later in life(Victora et al. 2016). Although clinically, breastfeeding may represent a vehicle for the transmission of infectious diseases to infants, including viral infections, its benefit typically outweighs any risk(Lawrence 2011). There are, however, circumstances when breastfeeding is contraindicated such as maternal infection with human immunodeficiency virus (HIV-1/2) or human t-lymphocytic virus (HTLV)-I/II in a developed country or herpes simplex virus with active lesions on the breast(Eidelman and Schanler 2012).

While their mother's own milk supply is being established, human donor milk is used as a bridge for hospitalized infants; 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). 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). Milk banking associations, including the Human Milk Banking Association of North America (HMBANA) and the European Milk Banking Association (EMBA) 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 undergoes low-temperature long-time pasteurization, known as the Holder method (62.5ºC, 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. 2018). It is also important to understand if other pasteurization methods can inactivate SARS-CoV-2, including high-temperature short-time, proposed as an alternative technique in human milk banking, in addition to flash-heating, a home-based method that takes place with informal milk sharing("Eats on Feets Resources-Resource for Informed Breastmilk Sharing" 2011).

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 from the mammary tissue, notably, cytomegalovirus, HTLV and HIV, or may be present as a contaminant from skin or respiratory droplets either in the milk or on collection containers(Michie 2001). Regardless of origin, accurate data is needed around thermal inactivation of viruses to avoid confusion and misinformation around the safety of human donor milk.

To date, there has been no systematic review of the impact of thermal pasteurization on viral load or detectable live virus 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. Macronutrient analysis was not

considered in association with viral load in any study and therefore, not considered as part of this review. The search strategy is summarized in Supplementary 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*=64,949 records were excluded on the basis of title and abstracts alone, encompassing articles that did not meet the inclusion criteria (*n*=44,286 records) or were duplicate records (*n*=20,663). After title and abstract screening, 182 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, 109 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 17 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. Since human milk alone may reduce viral load and detectable live virus, 2 studies reported diluting milk samples prior to assay and 6 studies controlled for the independent effect of human milk on reducing infectivity(Dworsky et al. 1982; Orloff et al. 1993; Terpstra et al. 2007; Volk et al. 2010; Hoque et al. 2013; Donalisio et al. 2014; Hamilton Spence et al. 2017; Pfaender et al. 2017). Predominantly, the viruses tested were caspid enveloped and belonged to 7 different viral families including: 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 ($TCID_{50}$) 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 62.5ºC - 63ºC 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 100ºC, 5 min(Welsh et al. 1979). In fact, for cytomegalovirus specifically, studies that demonstrated complete inactivation collectively required temperatures of 60ºC-63ºC for varying lengths of time (between 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 60ºC, for a minimum of 5 seconds. In particular, flash heating (at-home pasteurization method) 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 (72ºC 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-enveloped members of the picornaviridae family were found to be more resistant to heating(Terpstra et al. 2007); high-temperature short-time treatment (72 ºC for 16 seconds) of

hepatitis A virus and porcine parvovirus yielded a 2- or 0.5-log reduction in $TCID_{50}$/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 92 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, parvoviridae, 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=25*), 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 60ºC-65ºC for 10-180 min(Croci et al. 1999; Bidawid et al. 2000; Gibson and Schwab 2011); to 72ºC for 1-13 min (Bidawid et al. 2000; Araud et al. 2016), and to 90ºC 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

60ºC - 67ºC for 1-60 min (Gibson and Schwab 2011; Shao et al. 2018), >3.5-log reduction at 72ºC for 1 min (Hewitt et al. 2009; Araud et al. 2016), and >5-log reduction at 85ºC -90ºC for 1-5 min (Sow et al. 2011; Park et al. 2014a). HIV 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 60ºC-65ºC for 10-15 min (Lelie et al. 1987; Gregersen et al. 1989) ; similar reductions were observed at 77ºC-80ºC 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 in cell culture media and plasma products; complete inactivation was observed at temperatures between 56ºC-60ºC for a 5-60 min duration(Duan et al. 2003; Darnell et al. 2004; Yunoki et al. 2004; Kariwa et al. 2006; Chin et al. 2020). Other coronaviruses, including canine coronavirus, and MERS-CoV show sensitivities to heating in cell culture media, bovine milk or camel milk, where a clinically relevant reduction in infectivity (>4.5 – 5.5 log $TCID_{50}$) is attainable upon heating at 63ºC-65ºC for 5-30 minutes(Pratelli 2008; Leclercq et al. 2014; van Doremalen et al. 2014). Furthermore, cytomegalovirus, a member of the herpesviridae family was completely inactivated at temperatures between 50ºC-65ºC for 15-30 min(Plummer and Lewis 1965; Lelie et al. 1987; Fanner 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 50ºC and 65ºC 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 (62.5ºC, 30 min) is the most common method used(Arslanoglu et al. 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 62.5ºC 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 63ºC (70ºC -90ºC) or a significantly longer duration at 60ºC-63ºC (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. Thus clinically, standard pasteurization procedures conducted at milk banks should be adequate to ensure a safe supply of human donor milk. 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 investigation is warranted using standardized research methodology and human milk as the matrix. In addition to thermal pasteurization, research into novel and innovative pasteurization systems for human milk must also be studied to ensure they can be used to successfully inactivate potential viral pathogens.

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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Table 1. Summary of studies assessing the effect of heat, including Holder pasteurization, on viral inactivation in human milk

Family	Virus	Envelope	Pasteurization	Method	Result	Reference
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	$TCID_{50}$	>7-log $TCID_{50}$/mL (complete inactivation) at 72°C, 4 sec	(Terpstra et al. 2007)
	Zika virus	Yes	63°C, 30 min	$TCID_{50}$	>6-log $TCID_{50}$/mL reduction (complete inactivation) at 63°C, 30 min	(Pfaender et al. 2017)
Herpesviridae	Cytomegalovirus	Yes	55°C-72°C, 5 sec	$TCID_{50}$	>4-log $TCID_{50}$/mL reduction (complete inactivation) at 60°C, 5s	(Maschmann et al. 2019)
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)
Herpesviridae	Cytomegalovirus	Yes	62.5°C, 30 min	SEAP Reporter	Complete inactivation at 62.5°C, 30 min	(Donalisio et al. 2014)
Herpesviridae	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)
Herpesviridae	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)
Herpesviridae	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)
Herpesviridae	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)
Herpesviridae	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)
Herpesviridae	Herpes simplex 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, 30min; >7-log PFU/mL reduction (complete inactivation) at 100°C, 5 min	(Welsh et al. 1979)
Herpesviridae	Pseudorabies virus	Yes	72°C, 16 sec	$TCID_{50}$	>8-log $TCID_{50}$/mL reduction (complete inactivation) at 72°C, 4 sec	(Terpstra et al. 2007)
Papillomaviridae	Human papillomavirus	No	62.5°C, 30 min	SEAP Reporter	Complete inactivation at 62.5°C, 30 min	(Donalisio et al. 2014)
Parvoviridae	Porcine parovirus	No	72°C, 16 sec	$TCID_{50}$	<1-log $TCID_{50}$/mL reduction at 72°C, 16 sec	(Terpstra et al. 2007)
Picornaviridae	Coxsackievirus B4	No	(1) 56°C, 30 min; (2) 63°C, 30 min; (3) 100°C, 5 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	$TCID_{50}$	3.5-log $TCID_{50}$/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)
Retroviridae	HIV-1	Yes	55°C-70°C, time to max temperature	PBMC neutralization assay	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	>3.4-log copies/mL reduction (complete inactivation) after flash heating	(Israel-Ballard et al. 2007)
Retroviridae	HIV-1	Yes	72°C, 16 sec	$TCID_{50}$	>8-log $TCID_{50}$/mL reduction (complete inactivation) at 72°C, 4 sec	(Terpstra et al. 2007)
Retroviridae	HIV-1	Yes	56°C- 62.5°C, 12-15 min	RNA assay	>5-log copies/mL reduction (complete inactivation) after pasteurization	(Jeffery et al. 2001)
Retroviridae	HIV-1	Yes	62.5°C, 30min	$TCID_{50}$	>5.5-log $TCID_{50}$/mL reduction (complete inactivation) at 62.5°C, 30 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, $TCID_{50}$. 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	$TCID_{50}$	>5.5-log $TCID_{50}$/mL reduction (complete inactivation) at 85°C, 2 h	(Sauerbrei and Wutzler 2009)
Adenoviridae	Canine adenovirus	No	FBS	56°C, 15-45 min	$TCID_{50}$	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	$TCID_{50}$	0.91-log $TCID_{50}$/mL reduction at 65°C, 30 min; >3.5-log $TCID_{50}$/mL reduction at 75°C, 30 min;	(Welch et al. 2006)
Birnaviridae	Infectious pancreatic necrosis virus	No	Media	37.5°C-60°C; 0-20 h	PRA	4-log reduction PFU/mL at 60°C, 7 h; >7-log reduction PFU/mL (complete inactivation) at 60°C, 16 h	(Gosting and Gould 1981)
Caliciviridae	Canine calicivirus	No	Media	4°C-100°C, (wks/sec)	$TCID_{50}$	3-log $TCID_{50}$/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	35°C-70°C, 2 min	PRA	>4.5-log PFU/mL reduction at 60°C, 2 min; >5-log PFU/mL reduction (complete inactivation) at 65°C, 2 min.	(Topping et al. 2009)
Caliciviridae	Feline calicivirus	No	Media	4°C-100°C, (wks/sec)	$TCID_{50}$	3-log $TCID_{50}$/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	(steam, boil), 37 sec,180 sec	qRT-PCR	2-log RT-PCR U/mL reduction after boiling for 180 sec (max temp 90°C)	(Hewitt and Greening 2006)
Caliciviridae	Human norovirus	No	Media	60°C-90°C, 2 min	qRT-PCR	2-3-log IU/mL reduction at 90°C, 2 min	(Li et al. 2017)
Caliciviridae	Human norovirus	No	Media	100°C, 3min	qRT-PCR	>7.5-log reduction IU/mL (complete inactivation) at 100°C, 3 min	(Duizer et al. 2004)
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	>3.5-log PFU/mL reduction (complete inactivation) at 63°C, 5 min (water) 3.2-log reduction 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-	(Bozkurt et al. 2014b)

			blue mussel			log PFU/mL reduction (complete inactivation) at 72°C, 20 sec	
Caliciviridae	Murine norovirus	No	Media	60°C-85°C, 0-30 min	$TCID_{50}$	>3-log $TCID_{50}$/mL reduction at 60°C, 30 min; >7-log $TCID_{50}$/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	$TCID_{50}$	>5-log $TCID_{50}$/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	$TCID_{50}$	0.25-log $TCID_{50}$/mL reduction at 65°C, 30 min; 1.92-log $TCID_{50}$/mL reduction at 75°C, 30 min;	(Welch et al. 2006)
Coronaviridae	Canine coronavirus	Yes	Media	56°C-75°C, 0-60 min	$TCID_{50}$	>6.5-log $TCID_{50}$/mL reduction (complete inactivation) at 56°C, 60 min	(Pratelli 2008)
Coronaviridae	MERS-CoV	Yes	Media	56°C, 65°C, 0.5-120 min	$TCID_{50}$	>5.5-log $TCID_{50}$/mL reduction (complete inactivation) at 56°C, 60 min; >5.5-log $TCID_{50}$/mL reduction (complete inactivation) at 65°C, 15 min	(Leclercq et al. 2014)
Coronaviridae	MERS-CoV	Yes	Media/ bovine /camel/ caprine milk	63°C, 30 min	$TCID_{50}$	>5.5-log $TCID_{50}$/mL reduction (complete inactivation) at 63°C, 30 min (all products)	(van Doremalen et al. 2014)
Coronaviridae	Mouse hepatitis virus	Yes	Blood plasma	65°C, 0-10 h	$TCID_{50}$	>7-log $TCID_{50}$/mL reduction (complete inactivation) at 65°C, 15 min	(Lelie et al. 1987)
Coronaviridae	SARS-CoV	Yes	Media	37°C-75°C, 0-120 min	$TCID_{50}$	>6-log $TCID_{50}$/mL reduction (complete inactivation) at 67°C, 60 min	(Duan et al. 2003)
Coronaviridae	SARS-CoV	Yes	Media	56°C, 0-90 min	$TCID_{50}$	>7-log $TCID_{50}$/mL reduction at 56°C, 30 min; >7-log $TCID_{50}$/mL reduction (complete inactivation) at 56°C, 60 min	(Kariwa et al. 2006)
Coronaviridae	SARS-CoV	Yes	Plasma product	60°C, 120 min	$TCID_{50}$	>6-log $TCID_{50}$/mL reduction at 60°C, 30-60 min; >6-log $TCID_{50}$ reduction (complete inactivation) at 60°C, 60 min	(Yunoki et al. 2004)
Coronaviridae	SARS-CoV	Yes	Media	56°C-75°C, 0-90 min	$TCID_{50}$	>5-log $TCID_{50}$/mL reduction (complete inactivation) at 56°C, 20 min; >4-log $TCID_{50}$/mL reduction (complete inactivation) at 65°C, 10	(Darnell et al. 2004)

						min	
Coronaviridae	SARS-CoV	Yes	HSA	56°C,65°C, 0-120 min	$TCID_{50}$	>5-log $TCID_{50}$/mL reduction (complete inactivation) at 56°C, 20 min	(Darnell and Taylor 2006)
Coronaviridae	SARS-CoV-2	Yes	Media	56°C, 70°C, 1-30 min	$TCID_{50}$	>6-log $TCID_{50}$/mL reduction (complete inactivation) at 56°C, 30 min; >6-log $TCID_{50}$/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	$TCID_{50}$	>7-log $TCID_{50}$ reduction (complete inactivation) at 60°C, 3 min	(Madani et al. 2014)
Flaviviridae	Bovine viral diarrhea virus	Yes	FBS	56°C, 15-45 min	$TCID_{50}$	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	60°C-61°C, 10 h	$TCID_{50}$	>6.5-log $TCID_{50}$/mL reduction (complete inactivation) at 60°C, ≤ 2 h	(Plavsic 2000)
Flaviviridae	Bovine viral diarrhea virus	Yes	Diaspirin 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	60°C, 10 h	$TCID_{50}$	>6-log $TCID_{50}$/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	$TCID_{50}$	>4-log $TCID_{50}$/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	$TCID_{50}$	>7-log $TCID_{50}$/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	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	$TCID_{50}$	>4-log $TCID_{50}$/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- 2 h-media;	$TCID_{50}$	>5-log $TCID_{50}$/mL reduction (complete inactivation) at 56°C, 5 min	(Blümel et al. 2017)
Hepadnaviridae	Duck Hepatitis B virus	Yes	Hemoglobin solutions	60°C, 1 h	Bioassay	>6-log DIU/mL reduction (complete inactivation) at 60°C, 1h	(Fanner 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	(Song et al. 2011)
Hepeviridae	Hepatitis E virus	No	Fecal suspension	45-70°C, 1 h	IF	Complete inactivation (no Hepatitis E + cells) 60°C, 1 h	(Emerson et al. 2005)
Hepeviridae	Hepatitis E virus	No	Homogenized liver	62°C-71°C, 5-120 min	Bioassay	1.2-log reduction at 62°C, 5 min; 2.6-log reduction at 71°C 10 min	(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	>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)	(Imagawa et al. 2018)
Herpesviridae	Bovine herpes virus	Yes	Bovine serum albumin/transferri	60°C-61°C, 10 h	$TCID_{50}$	>4-log $TCID_{50}$/mL reduction (complete inactivation) at 60°C, ≤2h	(Plavsic 2000)

			n solution				
Herpesviridae	Bovine herpes virus	Yes	FBS	56°C, 15-45 min	$TCID_{50}$	Complete inactivation (reduction factor >27.24) at 56°C, 30 min	(Danner et al. 1999)
Herpesviridae	Bovine herpes virus	Yes	Immunoglobulin preparation	60°C, 10 h	$TCID_{50}$	4-log $TCID_{50}$/mL reduction at 60°C, 120 min; 5-log $TCID_{50}$/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	$TCID_{50}$	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	(Fanner et al. 1992)
Herpesviridae	Cytomegalovirus	Yes	Infant formula	62.5°C, 30 min	PRA	>3-log PFU/mL reduction (complete inactivation) at 62.5°C, 30 min	(Mikawa et al. 2019)
Herpesviridae	Duck plaque virus	Yes	Media	42°C-96°C, 2 h	$TCID_{50}$	>6-log $TCID_{50}$/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	Herpes simplex virus	Yes	Immunoglobulin preparation	60°C, 10 h	$TCID_{50}$	>5-log $TCID_{50}$/mL reduction at 60°C, 10 h	(Aghaie et al. 2008)
Herpesviridae	Pseudorabies 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)
Herpesviridae	Pseudorabies virus	Yes	Antithrombin III solution	60°C, 0-10 h	$TCID_{50}$	>7-log $TCID_{50}$/mL reduction (complete inactivation) at 60°C, 40 min	(Barrett et al. 1996)
Herpesviridae	Pseudorabies virus	Yes	Media	55°C-70°C, 0-15 min	$TCID_{50}$	>7-log $TCID_{50}$/mL reduction (complete inactivation) at 62°C, 10 min	(Turner et al. 2000)
Orthomyxoviridae	High pathogenicity avian influenza	Yes	Fat-free egg products	53°C-63°C, 0-40 min	$TCID_{50}$	>5-log $TCID_{50}$/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	$TCID_{50}$	>4-log $TCID_{50}$/mL reduction at 60°C, 2 min	(Chmielewski et al. 2011)
Orthomyxoviridae	Influenza virus	Yes	Allantoic fluid	46-54°C, 15 min	EID_{50}	Infectivity reduced significantly at 54°C, 15 min	(Chu 1948)
Orthomyxoviridae	Influenza virus	Yes	Allantoic fluid	56°C, 0-8 h	IV	>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	$TCID_{50}$	>3-log $TCID_{50}$/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	$TCID_{50}$	>5-log reduction $TCID_{50}$/mL 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	$TCID_{50}$	>5-log $TCID_{50}$/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_{50}	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_{50}	4-log EID_{50} reduction at 60°C, 10 min; >6-log EID_{50} reduction (complete inactivation) at 80°C, 10 sec	(Alexander and Manvell 2004)
Paramyxoviridae	Parainfluenza type 3 virus	Yes	FBS	56°C, 15-45 min	$TCID_{50}$	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	$TCID_{50}$	>2-log $TCID_{50}$/mL reduction 65°C, 40 min; 5.5-log $TCID_{50}$/mL reduction (complete inactivation) at 103°C, 90 sec	(Lelie et al. 1987)
Parvoviridae	Porcine parovirus	No	FBS	56°C, 15-45 min	$TCID_{50}$	Incomplete inactivation (reduction factor 1.09) at 56°C for 45 min	(Danner et al. 1999)
Parvoviridae	Porcine parovirus	No	HSA	60°C, 1-60 min	$TCID_{50}$	<1-log $TCID_{50}$/mL reduction at 60°C, 60 min	(Blumel et al. 2002)
Parvoviridae	Porcine parovirus	No	Diaspirin crosslinked hemoglobin	74°C, 90 min	PRA	>8.7-log PFU reduction (complete inactivation) at 74°C, 90 min	(Azari et al. 1998)
Parvoviridae	Bovine parvovirus	No	Media	40°C-95°C, 1-2 h	$TCID_{50}$	0.9-log $TCID_{50}$/mL reduction at 95°C, 2 h	(Sauerbrei and Wutzler 2009)
Parvoviridae	Minute virus of mice	No	Antithrombin III solution	60°C, 0-10 h	$TCID_{50}$	~3-log $TCID_{50}$/mL reduction at 60°C, 10 h	(Barrett et al. 1996)
Picornaviridae	Bovine enterovirus	No	Bovine serum albumin/transferrin solution	60°C-61°C, 10 h	$TCID_{50}$	>4-log $TCID_{50}$/mL reduction (complete inactivation) at 60°C, ≤2 h	(Plavsic 2000)
Picornaviridae	Encephalomyocarditis virus	No	Blood plasma	65°C, 0-10 h	$TCID_{50}$	>9-log $TCID_{50}$/mL reduction (complete inactivation) at 65°C, 15 min	(Lelie et al. 1987)
Picornaviridae	Encephalomyocarditis virus	No	Human plasma	60°C - 90°C, 0.25 sec	$TCID_{50}$	>5.8-log $TCID_{50}$/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	$TCID_{50}$	>3-log $TCID_{50}$/mL reduction at 55°C, 8 min; >5-log $TCID_{50}$/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	PRA	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	PRA	>7-log PFU/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	$TCID_{50}$	5-log reduction $TCID_{50}$/mL at 60°C, 120 min; 7-log $TCID_{50}$/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	PRA	>2-3-log PFU/mL reduction (complete inactivation) at 73°C, 18 sec	(Tomasula et al. 2007)
Picornaviridae	Foot and mouth disease virus	No	Bovine milk/cream	72°C, 0-5 min	PRA	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	$TCID_{50}$	≥ 3-log $TCID_{50}$/mL reduction at 62.8°C, 30 min (milk); 5-log $TCID_{50}$/mL reduction (complete inactivation) at 62.8°C, 30 min (PBS)	(Parry and Mortimer 1984)
Picornaviridae	Hepatitis A virus	No	Media	37°C-70°C, 5-60 min	$TCID_{50}$	1-log $TCID_{50}$/mL reduction at 50°C, 60 min; 4-log $TCID_{50}$/mL reduction 60°C, 60 min; >6-log $TCID_{50}$/mL reduction (complete inactivation) at 70°C, 30 min	(Flehmig et al. 1985)
Picornaviridae	Hepatitis A virus	No	0.1M NaCl or 2M $MgCl_2$	20°C, 60°C, 10 min	PRA	3.3-log PFU/mL reduction at 60°C,10 min	(Anderson 1987)
Picornaviridae	Hepatitis A virus	No	Media	60°C, 10 h	$TCID_{50}$	>3.6-log $TCID_{50}$/mL reduction (complete inactivation) at 60°C, 30 min	(Murphy et al. 1993)
Picornaviridae	Hepatitis A virus	No	Antithrombin III solution	60°C, 0-10 h	$TCID_{50}$	>6-log $TCID_{50}$/mL reduction (complete inactivation) at 60°C, 350 min	(Barrett et al. 1996)
Picornaviridae	Hepatitis A virus	No	Media/Mussel	(1) 60°C, 10-30 min; (2)	$TCID_{50}$	>5-log $TCID_{50}$/mL reduction at 60°C, 10 min	(Croci et al. 1999)

			homogenate	80°C, 3-15 min; (3) 100°C, 1-8 min		(media); >5-log $TCID_{50}$/mL at 60°C,15 min (homogenate)	
Picornaviridae	Hepatitis A virus	No	Bovine milk products	(1) 0-16 min, 65-70°C; (2) 0-5 min, 65-85°C	PRA	5-log PFU/mL reduction at 65°C, 41-46 min (all products); 5-log PFU/mL reduction at 73°C, 12-13 min (all products)	(Bidawid et al. 2000)
Picornaviridae	Hepatitis A virus	No	Fecal suspension	45-70°C, 1 h	IF	Complete inactivation (no Hepatitis A+ cells) at 66°C, 1 h	(Emerson et al. 2005)
Picornaviridae	Hepatitis A virus	No	Mussels	(steam, boil), 37 sec,180 sec	$TCID_{50}$	>2-log $TCID_{50}$/mL reduction (complete inactivation) at 180 sec boil (max temperature 90°C)	(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 (complete inactivation) at 63°C, 5 min (water); >3.5-log PFU/mL reduction (complete inactivation) at 63°C, 10 min (milk)	(Hewitt et al. 2009)
Picornaviridae	Hepatitis A virus	No	HSA	60°C, 0-10 h	IF	1-log FFU (infectivity) reduction at 60°C, 1 h; 3-5-log FFU reduction (infectivity)at 60°C, 10 h (strain-specific)	(Shimasaki et al. 2009)
Picornaviridae	Hepatitis A virus	No	Media	37°C-7°C, 180 min	PRA	4.5-log PFU/mL reduction 60°C, 180 min	(Gibson and Schwab 2011)
Picornaviridae	Hepatitis A virus	No	Green onions	45-65°C, 20 h dehydration	PRA	>2.4-log $TCID_{50}$/mL reduction in at temperatures >58°C	(Laird et al. 2011)
Picornaviridae	Hepatitis A virus	No	Soft-shell clams	85°C, 90°C, 90-300 sec	PRA	>2-log PFU/mL reduction at 85°C, 180 sec; >5-log PFU/mL reduction (complete inactivation) at 90°C, 10 sec	(Sow et al. 2011)
Picornaviridae	Hepatitis A virus	No	Mussels	Steam: 50°C -100°C	PRA	>3-log PFU/mL reduction (complete inactivation) after 6 min steam (~100°C max temperature)	(Harlow et al. 2011)
Picornaviridae	Hepatitis A virus	No	HSA	58°C, 600 min	$TCID_{50}$	3.1-5.2-log $TCID_{50}$/mL reduction at 58°C for 600 min (4.5-25% serum albumin)	(Farcet et al. 2012)
Picornaviridae	Hepatitis A virus	No	Manila clams	60°C, 10 min	$TCID_{50}$	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 at 65°C, 6 min	(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 at 60°C, 5 min	(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) 65°C/72°C, 0-90 sec	PRA	<1-log PFU/mL reduction 65°C, 90 sec; 1-log PFU/mL reduction 72°C, 60 sec	(Bozkurt et al. 2015a)
Picornaviridae	Hepatitis A virus	No	Media	(1) 62°C, 30 sec; (2) 72°C, 80 sec;(3) 80°C, 12 sec	PRA	>2-log PFU/mL reduction at 62°C, 30 min; >7-log PFU/mL reduction at 80°C, 12 sec	(Araud et al. 2016)
Picornaviridae	Poliovirus	No	0.1M NaCl or 2M $MgCl_2$	20°C, 60°C, 10 min	PRA	>4-log PFU/mL reduction (complete inactivation) at 60°C, 10 min	(Anderson 1987)
Picornaviridae	Poliovirus	No	Hemoglobin solutions	60°C, 0-10 h	PRA	>6-log PFU/mL reduction (complete inactivation) at 60°C, 30 min	(Estep et al. 1988)
Picornaviridae	Poliovirus	No	Immunoglobulin preparation	60°C, 10 h	$TCID_{50}$	>8-log reduction $TCID_{50}$/mL at 60°C, 10 h	(Aghaie et al. 2008)
Picornaviridae	Poliovirus	No	Media	60°C, 10 h	$TCID_{50}$	>5-log $TCID_{50}$/mL reduction (complete inactivation) at 60°C, 30 min	(Murphy et al. 1993)
Picornaviridae	Poliovirus	No	Bovine milk/water	(1) 62°C, 30 min;(2) 72°C, 15-30sec	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	$TCID_{50}$	>4.8-log $TCID_{50}$/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) 65°C, 0-10 h	$TCID_{50}$	>4-log $TCID_{50}$/mL reduction at 103°C, 90 sec (Lelie et al. 1987)
Polyomaviridae	Polyomavirus SV40	No	Media	40°C-95°C, 1-2 h	$TCID_{50}$	>5.1-log $TCID_{50}$/mL reduction (complete inactivation) at 95°C, 2 h (Sauerbrei and Wutzler 2009)
Poxviridae	Vaccinia virus	Yes	Blood plasma	65°C, 0-10 h	$TCID_{50}$	>5.8-log $TCID_{50}$/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	$TCID_{50}$	>4.3-log $TCID_{50}$/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	$TCID_{50}$	>7-log $TCID_{50}$/mL reduction at 60°C, 10 h (Aghaie et al. 2008)
Reoviridae	Reovirus	No	FBS	56°C, 15-45 min	$TCID_{50}$	Complete inactivation (reduction factor >5.50) at 56°C for 30 min (Danner et al. 1999)
Reoviridae	Avian Reovirus	No	Bovine serum albumin/transferrin solution	60°C-61°C, 10 h	$TCID_{50}$	>4-log $TCID_{50}$/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	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	37°C, 47°C, 62.8°C (milk only), 30 min	RT activity	Complete inactivation at 47°C/62.8°C, 30 min (Moore et al. 1996)
Retroviridae	Bovine leukemia virus	Yes	Media	56°C-73°C; 0.5-1 min	$TCID_{50}$	Complete inactivation at temperatures >60°C (Baumgartener et al. 1976)
Retroviridae	Bovine leukemia virus	Yes	Bovine milk	(1) 63°C, 30 min; (2) 72-73°C, 15-20 sec	Bioassay	Complete inactivation at 63°C, 30 min; Complete inactivation at 72°C -73°C, 15-20 sec (Chung et al. 1986)
Retroviridae	Human immunodeficiency virus	Yes	Blood plasma	65°C, 0-10 h	$TCID_{50}$	>4-log $TCID_{50}$/mL reduction (complete inactivation) at 65°C, 15 min (Lelie et al. 1987)
Retroviridae	Human immunodeficiency virus	Yes	Hemoglobin solutions	60°C, 0-10 h	PRA	Complete inactivation at 60°C, 7 min (Estep et al. 1988)
Retroviridae	Human immunodeficiency virus-1	Yes	Media	60°C, 10-240 min	$TCID_{50}$	5.5-log $TCID_{50}$/mL reduction (complete inactivation) at 60°C, 10 min (Gregersen et al. 1989)
Retroviridae	Human immunodeficiency virus	Yes	Hemoglobin solutions	60°C, 1 h	ELISA Early Ag	>4-log IU/mL reduction (complete inactivation) at 60°C, 30 min (Fanner et al. 1992)
Retroviridae	Human immunodeficiency virus-1	Yes	Antithrombin III solution	60°C, 0-10 h	$TCID_{50}$	>6-log $TCID_{50}$/mL reduction (complete inactivation) at 60°C, 20 min (Barrett et al. 1996)
Retroviridae	Human immunodeficiency virus-2	Yes	Media	60°C, 10-240 min	$TCID_{50}$	5-log $TCID_{50}$/mL reduction (complete inactivation), 60°C, 10 min (Gregersen et al. 1989)
Retroviridae	Human T lymphotropic virus III	Yes	Human plasma	60°C - 90°C, 0.25 sec	$TCID_{50}$	>4.4-log $TCID_{50}$/mL reduction (complete inactivation) at 77°C- 80°C, 0.25 sec (Charm et al. 1992)
Retroviridae	Human T lymphotropic virus III	Yes	Human serum	56°C, 0-30 min	$TCID_{50}$	>5-log $TCID_{50}$ reduction (complete inactivation) at 56°C, 10 min (Martin et al. 1985)

Retroviridae	Human T lymphotropic 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 lymphotropic virus III	Yes	Media/serum/ factor VIII	37°C-60°C, 0-120 min	$TCID_{50}$	>6-log $TCID_{50}$/mL reduction (complete inactivation) at 60°C, 2 min in all liquid matrices	(Mcdougal et al. 1985)
Retroviridae	Lymphadenopathy-associated virus	Yes	Media	37°C-56°C, 0-30 min	RT	63% inactivation at 48°C at 30 min; ~100% inactivation at 56°C at 20 min	(Spire et al. 1985)
Retroviridae	Murine leukemia virus	Yes	Blood plasma	65°C, 0-10 h	$TCID_{50}$	>5-log $TCID_{50}$/mL reduction (complete inactivation) at 65°C, 15 min	(Lelie et al. 1987)
Retroviridae	Rous sarcoma virus	Yes	Media	60°C, 10-240 min	$TCID_{50}$	4-log $TCID_{50}$/mL reduction (complete inactivation) at 60°C, 60 min	(Gregersen et al. 1989)
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	$TCID_{50}$	>3-log $TCID_{50}$/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	$TCID_{50}$	>4.4-log $TCID_{50}$/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	$TCID_{50}$	>6- log $TCID_{50}$/mL reduction at 60°C, 10 h	(Aghaie et al. 2008)
Togaviridae	Chikungunya virus	Yes	Various	56°C, 0-120 min	$TCID_{50}$	2.74-log $TCID_{50}$/mL reduction at 56°C, 15 min; >5-log $TCID_{50}$/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	$TCID_{50}$	>4-log $TCID_{50}$/mL reduction (complete inactivation) at 60°C, 5 min	(Franz et al. 2018)
Togaviridae	Mayaro virus	Yes	Various	56°C, 0-120 min	$TCID_{50}$	>2-log $TCID_{50}$/mL reduction at 56°C, 30 min; >5-log $TCID_{50}$/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	$TCID_{50}$	>10-log $TCID_{50}$/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 1963)

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.

Table 3. Comparing the log reductions in detectable live viruses pasteurized in both a human milk and a non-human milk matrix

		Human Milk Matrix				Non-Human Milk Matrix			
Family	Virus	Reduction*	Temperature†	Time‡	Reference	Reduction*	Temperature†	Time‡	Reference
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; Farcet 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; Fanner 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; Bidawid 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	Human immunodeficiency virus	>5.5	62.5	30	(Orloff et al. 1993)	>4	60-65	7-30	(Lelie et al. 1987; Estep et al. 1988; Gregersen et al. 1989; Fanner 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 $TCID_{50}$/mL

† Degree °C

‡ Minutes

Figure Caption

Figure 1. PRISMA flow diagram describing the selection of studies for inclusion in the review