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Review

Thermal inactivation of animal virus pathogens

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ABSTRACT

This article reviews available data and mechanisms regarding the thermal inactivation of a number of important pathogenic animal viruses in comparison with relevant surrogate viruses. Data obtained from over fifty years of research is reviewed with respect to heat inactivation conditions, testing methods, and the mechanisms of virus heat inactivation for the most significant animal virus pathogens. The mechanisms of heat inactivation are described based on fundamental studies of virus particle integrity derived from structural studies of virus cell entry and virus disassembly. The mechanism of the heat inactivation of the ssRNA poliovirus was determined from cell entry studies showing that Polio Virus (PV) heat treatment results in step-wise antigenic changes and eventual exposure of the viral nucleic acids without capsid disassembly. A similar model is apparent for the ssDNA parvovirus, however, these models differ from foot and mouth disease virus showing that thermal inactivation is accompanied by capsid disassembly. Cultured viruses when assayed in solution were generally inactivated by heating to 71 °C for one minute but may be more resistant to dry heat. In general enveloped viruses were more sensitive to heat inactivation than non-enveloped viruses. Amongst the most heat resistant viruses reported were the small DNA viruses including parvoviruses and circoviruses. The important enveloped animal virus pathogens such as highly pathogenic avian influenza and Newcastle disease virus were heat sensitive. However there were few data that showed a direct comparison between the inactivation of different viruses within the same matrix. Graphical representations of virus thermal inactivation kinetics usually show tailing effects and deviation from log-linear inactivation kinetics. Tailing may result from experimental error, virus aggregation, matrix effects, or the transfection of viral nucleic acids.

KEYWORDS: heat, thermal, thermo-stability, inactivation, infectivity, virus

ABBREVIATIONS

ELD, Embryonic Lethal Dose; CID, Chick Infectious Dose; TCID, Tissue Culture Infectious Dose; ND, Not Determined

INTRODUCTION

Data from virus heat inactivation studies are essential in ensuring that control measures for animal feed and food for human consumption are adequate for the prevention of disease and zoonotic transfer. This review spans more than 50 years of research and provides a summary of available data and mechanisms for the thermal inactivation of pathogenic animal viruses under heat inactivation conditions relevant to food processing. Food processing encompasses a variety of temperature and time treatments used individually or in combination. As a minimum, High Temperature Short Time (HTST) pasteurisation conditions for milk and fruit juices typically involve heat-treatment at 72 ºC for 15 s whilst those for Ultra High Treatment (UHT) involve heating at 135 ºC for 1-2 s. Many different food processes exist such as retorting, frying, boiling, heating, heating and extrusion, and

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Ultra High pressure (UHP) treatments. Traditionally, food microbiologists have sought to understand and model microbial inactivation kinetics by determining D-values (the time required for a 1 log reduction in infectivity at a given temperature) and Z-values (the temperature difference resulting in a 1 log reduction in D-value) in order to predict the inactivation of microbes using different temperature and time combinations. More recently D- and Z-values have been measured for a number of animal viruses. The thermal inactivation of viruses has been reviewed previously in [1, 2] and this review provides additional data and findings. Other useful reviews covering the survival of viruses under different conditions are also available, for e.g. [3, 4, 5, 6].

Animal–borne viruses of relevance to the food industry were reviewed in [6]; these included a number of viruses pathogenic to man and animals, and viruses with zoonotic potential as recognised by the World Organisation for Animal Health (OIE). These viruses are described and shown in Table 1 and include African Horse Sickness Virus (AHSV), African Swine Fever Virus (ASFV), Avian Influenza Virus (AIV), Bluetongue Virus (BTV), Classical Swine Fever Virus (CSFV), Foot and Mouth Disease Virus (FMDV), Infectious Bursal Disease Virus (IBDV), Newcastle Disease Virus (NDV), Peste des Petits Ruminants Virus (PPRV), Rift Valley Fever Virus (RVFV), Rinderpest Virus (RV), Swine Vesicular disease Virus (SVDV) and Vesicular Stomatitis Virus (VSV). Emerging pathogenic viruses (Circoviruses and Parvoviruses) are also included within this review for comparison, and to show reported inactivation mechanisms. Historically, most research studies have examined three important small single stranded RNA pathogens belonging to the *Picornaviridae* i.e., rhinoviruses, poliovirus (PV), and FMDV. More recently data has also been published for surrogate human norovirus pathogens that include the Feline Calicivirus (FCV) strain FCV F-9 and the Murine Norovirus (MNV) strain MNV-1. These viruses belong to the *Caliciviridae* and are included as model animal ssRNA viruses for comparison. Most data for important pathogenic enveloped viruses has been obtained from pathogenic AIV and NDV viruses.

Literature searches

Data for heat inactivation studies for this review were obtained predominantly from the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed) using the following search string with wildcards: virus AND inactiv* OR infect* OR destruction AND heat OR thermal OR temperature OR thermo*. This resulted in 10,076 citations up to 23rd March 2013, of which 2602 were related to the selected viruses. Many of the selected thermal inactivation

Table 1. Animal virus pathogens recognised by OIE.

Family	Virus	Genome	Size (kb)	Sense	Envelope
Asfaviridae	African swine fever virus	dsDNA	150-190	+	Yes
Birnaviridae	Infectious bursal disease virus	ds segmented RNA	6	+	No
Bunyaviridae	Rift valley fever virus	ss RNA segmented	11-15	-	Yes
Flaviviridae	Classical swine fever virus	ssRNA	13	+	Yes
Orthomyxoviridae	Avian influenza virus	ss RNA segmented	14	-	Yes
Paramyxoviridae	Newcastle disease virus	ssRNA	16	-	Yes
Paramyxoviridae	Peste des Petits ruminants virus	ssRNA	16	-	Yes
Paramyxoviridae	Rinderpest virus	ssRNA	16	-	Yes
Picornaviridae	Foot and mouth disease virus	ssRNA	7.5	+	No
Picornaviridae	Swine vesicular disease virus	ssRNA	7.5	+	No
Reoviridae	African horse sickness virus	ds segmented RNA	18	+	No
Reoviridae	Bluetongue virus	ds segmented RNA	19	+	No
Rhabdoviridae	Vesicular stomatitis virus	ssRNA	11	-	Yes

Data source: ICTVdB - The Universal Virus Database, version 4. http://ictvdb.bio-mirror.cn/ICTVdB/ICTVdB.htm#Begin

citations were not relevant to food processing and were related to low temperature vaccine stability studies or the elimination of viruses from blood products. Other irrelevant citations included those resulting from environmental studies, detection methods, prevalence or occurrence studies, descriptions of outbreaks, molecular epidemiology, treatment efficacy, immunology and fundamental studies of virus biology etc. Further literature searches also included high temperature short time (HTST), OIE guidelines, and grey literature relevant to virus inactivation in food processing. Some information gaps were evident for certain viruses and relevant citations for RVFV and PPRV were not found. However, enveloped PPRV belongs to the same *Paramyxoviridae* virus family as NDV.

The literature refers to a number of key variables in heat inactivation studies, which include: the virus, virus concentration, the particular strain, the test matrix, humidity (wet *versus* dry heat), pH and ionic strength, all of which can affect both the stability of the virus capsid, virus aggregation, and the accessibility of the virus particles to heat [1]. These factors are reviewed below. Inactivation data and citations for the animal pathogens and selected surrogate viruses are shown in Table 2 together with information related to the matrix and assays performed.

Measurement of virus heat inactivation

Assays for infectivity were generally measured by plaque assay or dilution against permissive cells to obtain the tissue culture infectious dose ($TCID_{50}$). Additionally, assays for avian viruses were performed in eggs referred to as the embryonic infectious dose (EID_{50}) or embryonic lethal dose (ELD_{50}). For FMDV further confirmation of loss of infectivity has also been based on animal studies. FMDV animal tests appear more sensitive than plaque assays and can apparently detect animal infection when not detectable by plaque assay [7]. There is a growing interest in the use of modified polymerase chain reaction (PCR) based methods to predict infectivity [8].

The literature showed considerable variation in the reporting of heat inactivation studies. Methods were often not described in detail, particularly with regard to ensuring that the sample reached the stated temperature for the required time. In some cases, e.g. long time periods, this is less relevant, however this needs to be determined accurately for high temperature short time processes. The presence of air and droplets adherent to tube walls, may contribute to non-uniform testing conditions and represent potential sources of error [9]. Heating methods used were either batch or continuous. Batch methods were most commonly represented by incubation of samples in glass vials, or capillary tubes in water baths, or more recently within tubes in PCR thermocyclers, and are typical of laboratory experiments. Continuous flow methods are also available and may be more representative of HTST treatments but are difficult to apply requiring specialist equipment [10]. Most studies of heat inactivation relevant to food processing have been carried out in suspension studies. However, it is worth noting that viruses are considerably more resistant to dry heat than to wet heat [11].

Inactivation studies and data were frequently obtained using a single cultivable strain of a virus species without comparison to a standard or to another virus. Direct comparison between viruses is difficult since different viruses are usually grown under different conditions. More direct comparison by spiking experiments is generally not performed in plaque assays owing to the possible risks from genetic recombination. It is therefore difficult to distinguish comparative effects between different viruses, unless particular attention is paid to virus purification and assay conditions. Only a few comparative virus inactivation studies have been performed simultaneously under the same conditions [12-15].

In some reports the same laboratory has published data for a number of different viruses. Lelie *et al.* [13] compared the heat inactivation of twelve different virus families in reconstituted human serum (0.6% v/v) under two of the steps used for the manufacture of Hepatitis B vaccine. Heating at 65 °C for 15 min completely inactivated (> 4 log reduction) nine of the twelve selected virus families. Only Canine parvovirus (CPV), Simian virus 40 (SV-40) and bacteriophage ØX174 survived. These viruses were also tested by heating at 93 °C for 90 s, resulting in the elimination of infectivity of bacteriophage ØX174 and that of CPV; however residual infectivity of SV-40 remained.

Differences have been demonstrated in the thermal tolerance of different mutants and strains of viruses e.g. for NDV [16] and for AIV strain H2N3 [17]. However this data was obtained based on low

Table 2. Thermal inactivation data for animal viruses.

Virus	Assay	Assay matrix	Results with log reductions	D-values	Z-values	Reference
Asfaraviridae						
African Swine Fever Virus (ASFV)						
AFSV 20/1	Haemadsorption	Pig slurry	> 4 log 65 °C 1 min	ND	ND	[64]
AFSV	Haemadsorption and immunosorbtion assays. Animal models.	Infected meat	Haemadsorption detected following low temperature < 35 °C processing for salami and pepperoni sausages but no infectivity detected.	ND	ND	[65]
AFSV	Plaque	Pig slurry and medium	> 5 log 70 °C 3 min	ND	ND	[55]
Birnaviridae						
Infectious Bursal Disease Virus (IBDV)						
IBDV 52/70	$TCID_{50}$	Bursal homogenate supernatant from infected chickens	4 log reduction	D_{80} = 180 s	ND	[66]
D78, STC, Del-A, MD & OH	$TCID_{50}$	Culture medium	1 min at 65, 71 or 100 °C results in > 1,> 2 or > 3 log reductions. Viable IBDV virus detected from cooked poultry at 71 or 74 °C for 6 min.	ND	ND	[67]
Flaviviridae						
Classical Swine Fever Virus (CSFV)						
CSFV		Contaminated meat	Inactivated at 65 °C 30 min or 71 °C 1 min	ND	ND	Reviewed by [68]
CSFV	$TCID_{50}$	Pig slurry or medium	> 5.0 log 70 °C 1 min. Less resistant than FMDV.	ND	ND	[55]

Table 2 continued..

CSFV	Plaque assay and animal models	Ham cubes	Inactivated 71 °C 1 min	ND	ND	[69]
Hog Cholera	Plaque assay and animal models	Infected meat	Survived low temperature < 35 °C processing for salami and pepperoni sausages.	ND	ND	[65]
Orthomyxoviridae						
Highly pathogenic (HP) and lowly pathogenic (LP) avian influenza viruses (AIV)						
HPAIV H5N2	ELD_{50}	Naturally infected chicken. Artificially infected chicken meat. Amniotic fluid	4-6 log	D_{61} = 24 s > resistance in naturally infected meat. Inactivation at 74 °C in < 1 s.	4.1 °C	[52]
HPAIV H5N2	ELD_{50}	Dried egg white, 7.5% moisture	> 4 log	D_{71} = 3000 s	18.2 °C	[70]
HPAIV H5N1	ELD_{50}	Naturally infected chicken	> 4 log	$D_{57\text{-}61}$ = 241-321 s D_{70} = 0.28 s D_{74} = 0.04 s	4.6 °C	[71]
HPAIV H5N1	ELD_{50}	Naturally infected chicken	> 4-5 log 70 °C 1 s	ND	ND	[72]
HPAIV H5N2 LPAIV H7N2	ELD_{50}	Artificially infected egg products	> 3 log	D_{63} = < 20 s in whole egg, liquid egg white, 10% salted yolk. May persist in dried egg white at 63 °C but < 1 day	1.6-9.0 °C depending on egg product	[73]
HPAIV H5N1	ELD_{50}	Infectious allantoic fluid	> 6-8 log 70 °C 10 min	ND	ND	[74]
LPAIV H7N7	Plaque assay	1% foetal calf serum or artificially infected chicken meat	> 4 logs 65 °C 30 s	ND	ND	[75]
HPAIV H5N2 LPAIV H7N2	TBC	Fat free egg product		$D_{55\text{-}59}$ = 1116-24 s $D_{55\text{-}59}$ = 174-30 s	4.4 °C 0.4 °C	[76]

Table 2 continued..

Paramyxoviridae						
Newcastle Disease Virus (NDV)						
NDV 24 different isolates	Plaque	Infectious allantoic fluid	2 log 56 °C 10 min	ND	ND	[77]
NDV/CA/02 NDV/Ulster	ELD_{50}	Naturally infected chicken meat and artificially infected chicken meat	4-6 log	D_{74} = < 0.1 s	3.7 - 4.8 °C	[52]
NDV/Ulster NDV/B1 NDV/CA/02	ELD_{50}	Artificially infected egg products	> 3 log	D_{63} = < 20 s in whole egg, liquid egg white, 10% salted yolk. May persist in dried egg white at 63 °C but < 1 day.	4.4 - 7.7 °C depending on egg product	[77]
NDV-cal.	ELD_{50}	Infectious allantoic fluid	6 log 60 °C 5 min	ND	ND	[78]
NDV Herts 33/56	ELD_{50}	Artificially infected chicken meat	6-8 log	D_{80} = 29 s	ND	[67]
NDV serotype 1	ELD_{50}	Fat free egg product		$D_{55\text{-}59}$ = 744-102 s $D_{55\text{-}63}$ = 744-10 s	4.7 °C 1.0 °C	[76]
Paramyxoviridae						
Rinderpest virus (RV)						
RV	CID_{50}	Tissues	Half-life 5 min 56 °C. Vaccine and virulent strains tested	ND	ND	[79]
Picornaviridae						
Foot and Mouth Disease Virus (FMDV)						
FMDV (Thailand) Serotypes O, A and Asia 1	$TCID_{50}$	Cultured virus in PBS	5-7 log Thai strains less heat resistant than other FMDV strains	D_{50} = 732 -1275 s D_{60} = 16 - 42 s D_{70} = 6 - 11 s D_{80} = 3 - 6 s D_{90} = 2 - 3 s D_{100} = 2 - 3 s D-values > D_{80} were less but not statistically different	19.9 - 21.2 °C	[80]

Table 2 continued..

FMDV Strain OBFS1860	Plaque	Pig slurry and medium	> 5 log 70 °C 3 min	ND	ND	[55]
FMDV Type O	ELISA & mouse and steer animal models	Milk from infected animals	> 4 log by ELISA. HTST temp ranges 72-95 °C for 19-36 s but animal infectivity remained. Protection by milk fat and proteins.	ND	ND	[10]
FMDV Type A_3	Plaque assay and steer animal models	Milk, skimmed milk, and cream from infected animals	> 4 log by culture but animal infectivity remained. Protection by milk fat and proteins e.g. casein and interferons. Survives in cream (93 °C for 15 s) and milk (72 °C for 15 s).	ND	ND	[53]
FMDV Type O_1	Steer animal models	Milk from infected animals	> 3.7 - 6.8 log Temp. ranges 80-148 °C. Inactivation requires 110 °C for 2 min or > 20 min at 100 °C.	ND	ND	[81]
FMDV Type O_1	Plaque and animal infectivity	Milk from infected animals	> 4 log reduction by culture following heat treatment at 72 °C for 12 s. Animal infectivity remained. Protection by milk fat and proteins. Freezing at -70 °C reduced temp sensitivity by 1 log.	ND	ND	[7]
FMDV (49 strains)	Plaque	Cultured virus in PBS	> 1.5 - 5.0 log following heat treatment at 54 °C for 1h.	ND	ND	[82]
FMDV RNA	Plaque assay / RNA transfection	Extracted RNA	Infectivity retained following 5 min at 100 °C. RNA protected by protein from inactivated virus.	ND	ND	[36]

Table 2 continued..

Swine Vesicular Disease Virus (SVDV)						
SVDV	Plaque assay	Pig slurry	> 6 log 65 °C 2 min	ND	ND	[83]
Reoviridae						
Blue Tongue Virus (BTV)						
BTV			< 3 log 56 °C 90 min	ND	ND	[84]
African Horse Sickness (AFHS)						
AFHS	Plaque assay	Vaccine from cell culture	> 5 log 36 °C for 40 days	ND	ND	[85]
Rhabdoviridae						
Vesicular Stomatitis Virus (VSV)						
VSV	$TCID_{50}$	Artificially infected plasma	> 4 log reduction 0.06 s at 75 °C	ND	ND	[63]
Emerging viruses						
Circoviridae						
PCV2 & CAV	Nested RT-QPCR	1:10 Bovine Serum Albumin	Resistant to dry heat 120 °C 3 log 30 min 80 °C	ND	ND	[59]
PCV2	RT-PCR Immuno-histochemistry	Cultured virus	Inactivated 15 min > 80 °C	ND	ND	[86]
PCV2 PCV1	$TCID_{50}$	Cultured virus	PCV 1 resistant to 15 min 70 °C. PCV2 > 5 log 95 °C 5 s	ND	ND	[62]
Parvoviridae						
PPV	CPE	Human milk	Resistant to 72 °C 16 s	ND	ND	[87]
Surrogate RNA viruses						
Picornaviridae						
Poliovirus (PV)						
Poliovirus	Plaque assay	Cultured PV	> 5 log 95 °C for 15 s in milk or water More resistant in milk.	ND	ND	[88]

Table 2 continued..

Poliovirus LS-a	Plaque assay	Cultured PV in PBS 2% collagen	> 6 log 80 °C 15 min > 6 log 121 °C 20 s			[58]
Poliovirus PV-1	Plaque assay / RNA transfection	RNA	Survived 60 °C for 1500 min.	ND	ND	[38]
Poliovirus PV-1	Plaque assay / RNA transfection	RNA	2.5 log reduction but RNA infectivity remained.	ND	ND	[37]
Poliovirus	Plaque assay / RNA transfection	RNA	RNA remained infectious after heating to 100 °C for 5 min.	ND	ND	[89]
Caliciviridae						
Murine norovirus MNV-1	Plaque assay	Cultured MNV -1	> 6 log 80 °C 2.5 min	ND	ND	[90]
Murine norovirus MNV-1	Plaque assay	Cultured MNV -1	5-6 log	D_{56} = 210 s D_{63} = 26 s D_{72} = 10 s	ND	[35]
Murine norovirus MNV-1	Plaque assay	Cultured MNV -1	4-5 log	D_{50} = 2069 s D_{56} = 219 s D_{60} = 18 s D_{72} = 9 s		[61]
Feline calicivirus FCV F-9	Plaque assay	Cultured FCV F-9	5-6 log	D_{56} = 405 s D_{63} = 24 s D_{72} = 10 s	ND	[35]
Feline calicivirus	Plaque assay	Cultured FCV F-9	4-5 log	D_{50} = 1214 s D_{56} = 382 s D_{60} = 34 s D_{72} = 6.7 s		[61]
Feline calicivirus FCV F-9	$TCID_{50}$	Cultured FCV F-9	3 log reduction 71 °C 1 min	D_{71} = 20 s	ND	[39]

temperature (55 °C) long time thermo-stability tests (primarily for vaccine stability). Thermostable variants are generally not present in FMDV quasi-species since higher structural stability appears to compromise virus uncoating and population survival [18]. However, thermostable variants have been observed for H2N3 [17]. Further studies may be required to test the relevance of data obtained from different mutants and strains when examining survival at higher temperatures.

The most widely reported recent studies on the inactivation of viruses in food and the environment have used NDV, Highly Pathogenic AIV (HPAIV) and FMDV owing to their prevalence and the potential consequences of infection. These studies have focused on the survival of viruses in meat, dairy products, animal feed, waste and the environment.

Mechanisms of heat inactivation

Loss of particle integrity

The mechanism of virus heat inactivation may vary at different temperatures. Dimmock N. J. [19] proposed (based on studies using rhinovirus and PV) that inactivation was a two component function. Inactivation may proceed through degradation of the viral nucleic acid at low temperatures (< 40 °C) or destruction of the virus coat protein and receptor binding at higher temperatures showing that heat treatment acts upon the virus capsid protein. Capsid stability and virus infectivity are not only influenced by temperature, but are also dependent on virus structure, pH, the particular strain, and purity of the test suspension [20-22]. Not surprisingly ionic strength also influences capsid stability depending on the selected virus [21, 23-25].

For PV, controlled heat treatment is used to model the stages of the infectivity process *in vitro* resulting in the egress of RNA [26]. Since egress of RNA also corresponds to loss of particle infectivity these studies also provide an insight into the steps that occur during the heat inactivation process. Heating of PV virus particles results in the conversion of mature virions (160S particles) via a 135S intermediate into 80S “empty” capsid shells without RNA. The 135S particles contain RNase resistant RNA and show externalisation of the normally internalized protein VP4 and externalization of the amino terminus of the major coat protein VP1. 135S particles also retain infectivity but show an altered cellular tropism for infection. Further heating of 135S particles results in the loss of VP4 and transition to 80S “empty” capsid shells. This transition is accompanied by expulsion or egress of part or all of the genomic RNA, resulting in 80S e (early, partially empty) or 80S l (late, completely empty) non-infectious particles. Viral RNA egress is thought to occur through a small pore in the capsid near a two-fold symmetry axis [26]. For PV, the loss of infectivity following heat treatment, the release of virus nucleic acid and antigenic conversion of capsid proteins occur simultaneously [27].

The process of PV infection as modeled by heat treatment appears similar to the heat inactivation model proposed for the single stranded DNA B19 parvovirus in which heat treatment also results in antigenic change accompanied by release of DNA [28]. In contrast, the capsid of the FMDV virus appears to dissociate into individual pentamers on heating and temperature-sensitive mutations affecting FMDV capsid stability map onto amino acids located within the interfacial pentameric sub-units [29]. FMDV capsid disintegration is also accompanied by the release of RNase sensitive RNA [20]. It therefore appears that for both RNA and DNA viruses heat inactivation results in the exposure of virus nucleic acid resulting in loss of infectivity either with or without the loss of complete capsid integrity. However, under mild heat conditions (such as those resulting in 135S particles) viruses may lose infectivity through loss of receptor binding without loss of viral RNA [30, 31].

Difference in the thermal uncoating of FMDV and PV may reflect the fundamental differences between enterically infecting PV and respiratory-infecting FMDV. Cellular uptake of the FMDV surrogate equine rhinitis A virus, human rhinovirus and FCV F-9, all proceed through clathrin–mediated endocytosis resulting in the dissociation of the capsid in the acidic pH of the endosome [32, 33]. In contrast, the cell entry of PV and the enteric norovirus surrogate MNV-1 is mediated through a non-clathrin, non-caveolae pathway [34]. Both enterically infecting PV and MNV-1 are more acid resistant than respiratory-infecting FMDV and the less closely related, norovirus surrogate FCV F-9 [25, 35]. The differences observed in the cell entry mechanisms and capsid dissociation may be explained by the

fact that FCV-F9 and FMDV capsids do not need to withstand transit through the acidic pH of the stomach. For viruses such as HPAIV and NDV the significantly reduced thermal stability in comparison to picornaviruses presumably results from a greater heat sensitivity of the lipid envelope compared with the virus capsid.

Nucleic acid stability

Although capsid destabilization results in loss of infectivity following heat treatment, RNA extracted from heat inactivated FMDV [36], PV or coxsackievirus B-2 virus particles remains intact and infectious in transfection assays, showing that heat treatment acts upon the virus capsid [37, 38]. Expelled nucleic acid is not completely "naked" as determined by partial resistance to RNase treatment and consists of nucleic acid complexed with protein as a ribonucleoprotein complex [8]. This apparently protects viral RNA from heat treatment compared to purified nucleic acid [19, 36]. In general terms, virus nucleic acid is resistant to thermal processing although natural transfection *in vivo* is unlikely to occur owing to the presence of nucleases within the environment. RT-qPCR signals derived from human Norovirus subjected to heating at 100 ºC for 1 minute remain relatively unaffected by heat treatment (ΔCq = 1.9) [39] showing that heat treatment alone has little effect on RT-qPCR signals. Low temperature virus inactivation (< 40 ºC) can result from single strand breaks caused by the enzymatic degradation of genomic RNA by internalised capsid enzymes including ribonuclease [40-42] or polymerase [43]. Nucleic acid stability is also a function of pH. High pH may result in denaturation of double stranded (ds) virus nucleic acid or degradation of RNA [44]. Single stranded (ss) RNA is less stable than dsRNA, ssDNA or dsDNA.

The tailing effect

A tailing effect is often observed in the graphical representation of virus inactivation data resulting in biphasic inactivation curves and "tail" of virus particles apparently resistant to inactivation. Tailing is frequently evident when large number of particles (> 10^5/ml) are present. Tailing may result from virus aggregation, micro-heterogeneity within the population, or may be indicative of different mechanisms of inactivation. Both virus concentration and storage conditions have been shown to influence virus aggregation and survival in variola virus [45] and vaccinia virus [46]. pH may also contribute to aggregation dependent on the pKa of the virus and the pH of the test matrix [47]. Micro-heterogeneity within the population does not appear to account for biphasic inactivation curves [48], although this has not been studied for many viruses. The occurrence of tailing effects at high virus concentrations may also result from experimental conditions including aggregation and uneven heat distribution [9]. Tailing has also been attributed to transfection of nucleic acids released by the heat treatment. Certain viral nucleic acids e.g. PV and FMDV can be transfected into cells under appropriate conditions at a low frequency (1/1000 – 1/100,000), resulting in infectious particles, suggesting that low level transfection may account for tailing effects observed in heat inactivation experiments at high virus titres [38, 48, 49]. Virus heat inactivation curves that show tailing effects that do not fit log-linear models can be more accurately modeled using probabilistic Weibull models of population survival, e.g. [50].

Matrix effects

Matrix effects can confer on viruses increased heat resistance or susceptibility, depending on the virus and the particular matrix. For PV it has been shown that matrix components such as fats, in particular myristate, and Hofmeister salts e.g. $MgCl_2$ appear to stabilize the capsid resulting in increased thermostability. The effect of salts was considered owing to the binding of anions and cations to charged residues in the protein molecule and/or the restructuring of water. In contrast, fats appeared to exert a specific effect by binding to the VP1 pocket, resulting in increased thermal stability of the capsid [51].

Viruses usually demonstrate increased thermal resistance when present in naturally contaminated samples or food matrices compared with tissue cultured viruses. HPAIV viruses present in artificially contaminated chicken meat are completely inactivated (> 4 log ELD_{50}) by incubation at 57 °C, but could still be isolated from naturally contaminated meat following incubation at 57 °C for 6 min However, there was little difference in the thermal inactivation of NDV when present in artificially infected or naturally infected chicken meat [52]. FMDV is more resistant to thermal inactivation when present in cream compared with milk, and appears to retain infectivity in animals following heat treatment at

93 °C for 15 s although infectivity in plaque assays was not detected [53]. Similarly Hepatitis E, derived from infected liver when present in experimentally contaminated food was found to require heat treatment at 71 °C for 20 min in order to abolish infectivity in a pig model [54]. In contrast African swine fever virus and swine vesicular disease virus are inactivated at a faster rate in pig slurry compared with buffered medium [55].

The capsid stability of FCV F-9 was significantly increased when added to dilute (1%) stool samples [56]. In contrast, no difference was found in the thermal stability of cultured PV when spiked in faeces [57]. Tuladhar *et al*. [15] recently reported similar findings for PV and MNV-1. These effects may be virus specific [12] and also dependent on virus concentration and aggregation, explaining discrepancies between studies.

PV may withstand heating at 121 °C for 15 s and shows increased heat resistance in the presence of collagen [58]. The heat resistant porcine circovirus PCV2 was more resistant in albumin than in human factor VIII when subjected to wet heat at 80 °C [59]. Similarly B19 parvovirus was more heat resistant in citrate buffer than in phosphate buffered saline (PBS), preventing virus inactivation by DNA externalization [60].

Kinetics of heat inactivation

The kinetics of virus inactivation has been reviewed (Hiatt, 1964). Historically, virus heat inactivation kinetic models have been based on first order reaction kinetics typically resulting in log-linear inactivation plots with temperature. However as noted above, plots of inactivation against temperature are frequently biphasic owing to the observation of tailing. Comparatively few studies have reported D-values and less frequently Z-values. The calculation of D- and Z-values from non-linear plots is difficult and therefore alternative models have been proposed to fit inactivation curves including Weibull models. In some cases there is little difference in D-values obtained from log-linear and Weibull plots [35, 61]. In other instances Weibull models appear to provide more accurate model inactivation for heat inactivation data [12, 50]. The use of different data interpretation appears dependent on the observation of a tailing effect which is dependent on the properties of the virus, virus concentration, pH and aggregation (as explained above).

Thermal inactivation data for pathogenic animal viruses

A general description of each of the selected viruses recognized as significant pathogens by OIE is given in Table 1. The inactivation temperatures quoted in the virus descriptions below are from the information provided on OIE information cards. Further information regarding the viruses and other animal pathogens may be found at: http://www.oie.int/en/our-scientific-expertise/specific-information-and-recommendations/technical-disease-cards/, the OIE web site http://www.oie.int/ and the Merck manual http://www.merckmanuals.com/vet/generalized_conditions.html. More detailed information regarding the viruses and their taxonomy is available at http://ictvonline.org/. Available thermal inactivation data extracted from citations is reported in Table 2 and is described below.

Asfaviridae

African swine fever virus (ASFV)

ASFV causes disease in pigs. ASFV may be spread by direct transmission through the oro-nasal route, ingestion of contaminated tissues and by tick vectors of the genus *Ornithodoros*i. ASF has been reported in a large number of countries in Africa, south of the Sahara, either as an endemic disease or as sporadic epidemics in domestic pigs. The disease has caused outbreaks in Europe and elsewhere and had become endemic in some European countries prior to the introduction of successful eradication programmes. The virus belongs to the *Asfaravidae* family, is enveloped and possesses a large (150-190 kbp) double stranded DNA genome. The virus is known to persist within the environment at low temperatures, but can be inactivated by heating to 56 °C for 70 min or 60 °C for 20 min. A greater than 5 log reduction occurred after heating the virus in pig slurry or medium at 70 °C for 3 min.

Birnaviridae

Infectious bursal disease virus (IBDV)

IBDV infection can result in a highly contagious disease of young chickens and causes immunosuppression and morbidity. IBDV is a member of the family *Birnaviridae*. These viruses have bipartite dsRNA genomes (A, 3.2 kb and B, 2.9 kb) enclosed in single-layered icosahedral capsids. The genomes may exist in a polyploid state. IBDV appears

heat resistant with a reported D-value of 3 min at 80 °C.

Bunyaviridae

Rift valley fever virus (RVFV)

RVFV causes an acute zoonotic disease of domestic ruminants in Africa and recently also in the Arabian peninsula. RVFV is vector-borne and predominantly spread by Aedes sp. mosquitoes and may also infect humans. RVFV virus belongs to the genus *Phlebovirus*, and is a typical Bunyavirus. RVFV is an enveloped virus and possesses a 3-segmented, negative-sense ssRNA genome with a molecular weight of 4-6 × 10^6. Each of the segments, L (large), M (medium), and S (small), is contained in a separate nucleocapsid within the virion. The virus is recoverable from serum after incubation at 56 °C for 120 min.

Flaviviridae

Classical swine fever virus (CSFV)

CSFV or Hog Cholera virus is an important pathogen of domestic pigs. Together with bovine viral diarrhea virus (BVDV) and border disease virus (BDV), CSFV belongs to the genus *Pestivirus* within the family *Flaviviridae*. The genome is a single positive stranded RNA of approximately 12.3 kb. The virus is readily inactivated by cooking e.g. heating meat to 65.5 °C for 30 min or 71 °C for one min (Table 2). OIE recommendations (2008) include heating meat in a hermetically sealed container to a minimum temperature of 70 °C throughout the meat. Other recommendations exist for fermented and cured products.

Orthomyxoviridae

Highly pathogenic avian influenza virus (HPAIV)

HPAIV is the causative agent of bird flu. HPAIV is represented by sub types H5 and H7. HPAIV belongs to the family *Orthomyxoviridae*, genus *Influenzavirus* A. The virus is enveloped and harbours eight segments of single-stranded genomic RNA of negative sense totalling approximately 14 kb. Transmission in birds occurs primarily through the faecal-oral route. The virus may be present in infected poultry at high concentrations. The virus is sensitive to wet heat and is readily inactivated in meat by heating to 74 °C for 3.5 s. The virus may persist for long periods in the environment, especially when present in faeces. Zoonotic transmission and evolution of virulence from lowly pathogenic AIV (LPAIV) to HPAIV is well documented. OIE recommendations for heat inactivation require heat treatment for 188 s at 60 °C for whole egg and 54.4 °C for 21.38 days for egg white. Meat should be cooked at a minimum temperature of 70 °C for 3.5 s.

Picornaviridae

Foot and mouth disease virus (FMDV)

FMDV is a positive sense single stranded RNA of 7.5 kb virus belonging to the family *Picornaviridae*. FMDV is endemic in the Middle East, Iran, the southern countries of the former Soviet Union, India, and south-east Asia. Europe is currently free from FMDV. All excretions and secretions from the infected animal contain virus, and virus may be present in milk and semen for up to 4 days before clinical signs appear. Conditions for inactivation have been extensively studied. The heat resistance of FMDV in contaminated products obtained from naturally infected cows is reflected in the OIE recommendations (2008) for FMDV inactivation. Meat processing requires the application of a minimum temperature of 70 °C for 30 min. Milk for human consumption requires an UHT process of 132 °C for 1 s, or if the pH is less than 7.0, the use of a HTST pasteurization step at 72 °C for 15 s. Alternatively, if the pH is greater than 7.0, then the HTST process must be applied twice. Milk for animal consumption requires two applications of the HTST step, or the use of additional process steps.

Swine vesicular disease virus (SVDV)

SVDV can cause an infectious disease in pigs resulting in symptoms similar to FMDV. SVDV is a single stranded RNA enterovirus within the *Picornaviridae* family. SVDV is most closely related to coxsackie B viruses with similar genome size and genetic organisation.

Paramyxoviridae

Newcastle disease virus (NDV)

Newcastle disease is a highly contagious and pathogenic disease of avian species. NDV is a type species of avian paramyxoviruses belonging to the genus *Avulavirus* and possesses a negative sense single stranded linear RNA of approximately 16 kb. The virus is enveloped and can survive long periods at ambient temperature when present in faeces.

The virus is inactivated by heating to 56 °C for 3 hours or 60 °C for 30 min. Reported D- and Z-values show that the virus is relatively heat sensitive, (Table 2).

Pest des petits ruminants virus (PPRV)

PPRV is classified in the *Morbillivirus* genus of the *Paramyxoviridae* family. This genus includes the measles type virus (MV) as well as a number of other animal pathogens including rinderpest virus (RPV), canine distemper virus (CDV) and dolphin distemper viruses that infect marine mammals. Morbilliviruses are non-segmented, negative sense ssRNA viruses with genomes approximately 15-16 kb in size. PPRV causes disease in sheep and goats with high mortality and was originally isolated in sub-saharan Africa. More recently there has been an increased global incidence with outbreaks in Turkey and India. The virus is destroyed by heating to 50 °C for 60 min.

Rinderpest virus (RV)

RV is classified in the *Morbillivirus* genus of the *Paramyxoviridae* family and is related to PPRV and canine distemper virus with a similar structure and genetic organisation. RV causes a highly fatal disease of cattle in immune susceptible populations. Small amounts of virus resist 56 °C for 60 minutes or 60 °C for 30 min.

Reoviridae

African horse sickness (AHSV)

AHSV is related to blue tongue virus (BTV) and possesses similar genomic organization also comprising 10 dsRNA genome of 18.5 kb segments within a double layered capsid. AHSV causes an acute or subacute, insectborne, viral disease of Equidae that is endemic to Africa. The virus is also spread by *Culicoides* biting midges. The presence of antibodies in other animals suggests that other animals may become infected and asymptomatic infection in dogs has been recognized. The virus is heat stable in plasma and retains infectivity after heating at 55-75 °C for 10 min.

Bluetongue virus (BTV)

BTV causes disease in ruminants and is spread by *Culicoides* biting midges but may also infect dogs and other carnivores. Secondary vector transmission may become significant through imbibing of blood from infected vertebrates. BTV is not contagious and concentrations in secretions and excretions have been reported as minimal. BTV possesses a double stranded segmented RNA genome and is the type species of the genus *Orbivirus* in the family *Reoviridae*. The genome comprises 10 dsRNA genome segments within a double layered capsid. BTV is inactivated by heating to 60 °C for 15 min.

Rhabdoviridae

Vesicular stomatitis virus (VSV)

VSV typically causes disease in cattle, horses, and pigs. Zoonotic and serologic evidence for infection exists in a number of other animals and birds. VSV is a viral disease caused by 2 distinct serotypes of vesicular stomatitis virus, New Jersey and Indiana. VSV is a negative sense ssRNA virus of approximately 11 kb. The viruses are members of the family *Rhabdoviridae* and genus *Vesiculovirus*. VSV viruses are the prototypes of the *Vesiculovirus* genus. VSVs are bullet shaped and generally 180 nm long and 75 nm wide. VSV is inactivated by heat treatment at 58 °C for 30 min.

Emerging pathogens

Circoviridae* and *Parvoviridae

In addition to the viruses listed by OIE, certain other animal pathogens including circoviruses and parvoviruses have emerged as heat resistant pathogens.

Circoviruses include the porcine circovirus (PCV1 and PCV2), and chicken anemia virus (CAV). These viruses are small (17 nm and 28 nm) and consist of covalently closed ssDNA genomes of 1.8 kb and 2.5 kb respectively. Data using PCR-based measure of *in vitro* replication suggest that these viruses are resistant to dry heat at 120 °C and appear more resistant to wet heat at 80 °C (2-3 log reduction after 30 min) than most other viruses [62].

The Parvovirus genus is 18-26 nm in diameter and possesses a linear ss DNA genome of approximately 5 kb with hairpin ends. The genus exists within the *Parvoviridae* family that includes a number of important pathogens including canine parvovirus.

Parvoviruses and circoviruses appear highly thermostable withstanding heat treatment at 70 °C for 15 min without reduction in infectivity. Heat treatments as high as 95 °C for 5 s are required to

inactivate (> 5 log reduction) PCV2 [63]. "Significant" reductions in porcine parvovirus (PPV) may occur following treatment at 90 °C for 0.006 s based on microwave heating in a "thermalyser" HTST device [63].

Data from surrogate RNA viruses

In many cases, data for pathogens is difficult to obtain owing to the containment conditions required for laboratory studies. This necessitates the use of attenuated vaccine strains or alternative surrogate viruses. Most commonly used small model RNA viruses include PV vaccine strains, FCV and MNV as shown in Table 2. Typical D-values for MNV and FCV at 72 °C were 10 s.

CONCLUSIONS

As a general rule, viruses, when grown in culture, are typically inactivated (> 4 log reductions) by heat treatment at 71 °C for one minute "*when normally expected levels*" of contamination occur [2], Table 2 confirms this, and shows that for the small ss RNA viruses such as FMDV, MNV-1 and FCV reported D-values at 70-74 °C are typically 10 s. Although enveloped viruses such as AIV and NDV appear comparatively more sensitive to heat inactivation with D-values of <1 s at 74 °C, measured D values may be much greater in food matrices with low moisture content as shown for HPAIV H5N2 when present in egg white at 7.5% moisture (D_{71} = 3000 s). Emerging virus pathogens such as the small ss DNA viruses appear the most resistant and in general, virus size appears inversely proportional to thermal stability. However thermal stability in solution appears constrained by the need for viruses to uncoat and release viral nucleic acid during the course of infection. Data for viruses when naturally present in animal tissues appears more variable when compared with viruses exhibiting similar or increased resistance in natural matrices. Differences associated with matrices may be more significant than that observed between different viruses; however comparative analysis of data is confounded by the large number of experimental variables that exist between published studies.

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CONFLICTS OF INTEREST STATEMENT

None

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