Literature search of heat and acid inactivation parameters of viruses relevant for food waste treatment for recycling to pig feed

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Authors

Hasmik Hayrapetyan[1], Masja Nierop Groot[1], Marcel Zwietering[2]

[1]Wageningen Food & Biobased Research (WFBR), Wageningen University & Research. Bornse Weilanden 9, 6708 WG Wageningen, The Netherlands.

[2]Laboratory of Food Microbiology, Wageningen University. Bornse Weilanden 9, 6708 WG Wageningen, The Netherlands.

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List of abbreviations

IPN	-	Infectious Pancreatic necrosis
APHA	-	UK Animal and Plant Health Agency
FMD	-	Food and Mouth Disease
AI	-	Avian Influenza
ND	-	Newcastle Disease
PED	-	Porcine Epidemic Diarrhoea
ASF	-	African Swine Fever
PRRS	-	Porcine Reproductive and Respiratory Syndrome
CSF	-	Classical Swine Fever

1 Introduction

Using food waste for animal feed provides sustainability and economic benefits and is currently practiced in Japan, US and South Korea. However, in the European Union (EU), food waste (potentially) containing products of animal origin are prohibited from being fed to livestock due to potential risks of spreading exotic diseases. The risks that may arise from such practices have been quantified by the UK Animal and Plant Health Agency (APHA) and reported in a risk assessment study (Adkin et al. 2014). In the report, heat inactivation is analysed as a potential risk mitigation strategy given that recontamination from untreated product streams is prevented. In the APHA study, three scenarios for heating were considered: 1) Processing 70°C/30 min, 2) Processing 100°C/1 hour and 3) Processing 130°C/30 min. These parameters were selected based on current practices in Japan and the US. To better understand how heat processing criteria may affect the risk, more information on heat inactivation parameters for relevant viruses is required, influence of the product matrix composition (e.g. liquid or dry product, pH). Moreover, some viruses are pH sensitive and a sufficiently low pH prevents germination and outgrowth of bacterial spores. Therefore, acidification by fermentation or by addition of acids may be useful as additional mitigation strategy.

This report describes the results of a short study to analyse available heat and acid inactivation data for swine and avian viruses relevant for safe use of the food waste as feed. This will provide insight in global temperature/time boundaries required to reach a desired level of inactivation of viruses – proportional to the risk. Moreover, it will help to decide on additional mitigation steps required to control viruses but also other (bacterial) pathogens, spores or contaminants (toxins). This study is based on a literature review of available data but is not meant to draft a design for a process, clearly many more factors including matrix composition, time to feeding etc. should be covered to come to a solution that covers all relevant pathogens and not only viruses. The study aims to provide insight in minimal process boundaries that would be required for mitigation strategies based on heat and/or acid and can aid in cost-effectiveness calculation for economic impact foreseen in Task 6.4 of REFRESH.

The aim

The aim of this study was to construct a database with heat and acid inactivation parameters for viruses – as a relevant group of pathogens for animal feed- and to extract global D and z-values[1]. These values can be used to understand the level

[1] D-values are the decimal reduction times, the time to reduce the organisms by a factor 10 or to 10%. z-values give the temperature dependence of the D-value.

of heat/ time or acid/time combinations minimally required to reach desired inactivation levels.

The microorganisms have been selected based on the outcome of the APHA risk assessment, for the scenario without segregation by species. Those hazards (viral diseases) which represent non-negligible risk in case of the mildest heat treatment in the study (70°C/30 min) were selected for this study (Table 1), even though the other pathogens may still be relevant to consider also. The focus of this literature search is on swine and avian viral diseases.

Also not included in this study (but still relevant to consider in eventual design of conditions for treatment of animal feed) are:

- Bacillus anthracis spores and the viral disease of fish (Infectious pancreatic necrosis (IPN)) are not included.
- During the REFRESH "Expert seminar on food waste treatment and risk management" in Wageningen (22 Nov 2017) several non-spore forming bacterial zoonosis were also proposed as hazards, most of which however are included in a study for global heat inactivation parameters by Van Asselt and Zwietering (2006), and were therefore not included in the current study.
- Spores of pathogenic Bacillus or Clostridium species are highly heat resistant and 70°C treatment will not inactivate them. These spores are also relevant for the food chain for human consumption, and requirements for food chains should also be considered for waste streams intended for animal feed.

Heat and acid inactivation parameters retrieved from scientific literature for the viruses listed in Table 1 will be collected in a database. Provided that enough inactivation data is available, these data can be modelled to obtain global D (time required for 1 log reduction) and z-value estimates.

Table 1. Viruses relevant for viral diseases that represent non-negligible risk in case of the waste heat treatment at 70°C/30 min and without segregation by species. The list is based on Table 10 of the APHA Risk Assessment on page 54*

Abbreviation	Full name of the virus	Species affected	Heat inactivation assumed in the risk assessment
HP-PRRS	Highly Pathogenic Porcine Reproductive and Respiratory Syndrome	Swine	1-log reduction at 70°C/30 min, 15 log at 100°C/60 min
ASF	African Swine Fever	Swine	Inactivated at 70°C/30 min
HP-PED	Highly pathogenic Porcine Epidemic diarrhoea	Swine	Inactivated at 70°C/30 min
CSF	Classical Swine Fever	Swine	Inactivated at 70°C/30 min
FMD	Foot and Mouth Disease	Multiple	5-log reduction at 70°C/30 min
HP-AI	Highly Pathogenic Avian Influenza	Avian	2.5 log reduction at 70°C/30 min
ND	Newcastle Disease	Avian	4 log reduction at 70°C/30 min

*Only swine and avian viral diseases were included. The fish disease (IPN) Infectious pancreatic necrosis was not included in the current study.

2 Materials and methods

2.1 Literature search in Scopus and Web of Science databases

Search purpose

Published articles describing heat inactivation or acid resistance/inactivation parameters for the selected viruses (Table 1).
Two databases to retrieve scientific literature were used: Scopus and Web of Science.

Searches for data for heat and acid inactivation of the relevant viruses

The search string contains terms for: inactivation method + inactivation+ the disease + virus + words for excluding the non-relevant articles.

Search 1. Scopus

TITLE-ABS-KEY ((heat* OR therm* OR temperature OR acid* OR " pH" OR ferment*) AND (inactiv* OR kill* OR sensitiv* OR resist* OR surviv* OR *stabil* OR eliminat* OR treatment OR persist* OR destruct* OR reduc*) AND ("Porcine Reproductive and Respiratory" OR "African Swine Fever" OR "Porcine Epidemic diarrh*" OR "Classical Swine Fever" OR "Foot and Mouth Disease" OR "Avian Influenza" OR "H5N1" OR "Newcastle Disease")) AND (virus* OR viral) AND NOT TITLE-ABS-KEY (vaccin* OR pathogenesis OR hemagglutinin OR haemagglutinin OR pathway OR immun* OR interferon OR cytotox* OR lymphocyt* OR macrophag* OR antigen OR virulence OR antibod* OR phage OR {amino acid} OR {nucleic acid} OR antibiotic OR neuraminidase OR inhibitor OR "plant extract" OR intravenous OR embryo OR "human infection" OR recombinant OR clinical OR loop-mediated OR "rapid detection" OR peptide OR gene) AND NOT TITLE (outbreak) AND (LIMIT-TO (LANGUAGE , "English"))

Search result: 380 hits

Search 2. Web of Science

(TS=(heat* OR therm* OR temperature OR acid* OR " pH" OR ferment*) AND TS=(inactiv* OR kill* OR sensitiv* OR resist* OR surviv* OR *stabil* OR eliminat* OR treatment OR persist* OR destruct* OR reduc*) AND TS=("Porcine Reproductive and Respiratory" OR "African Swine Fever" OR "Porcine Epidemic diarrh*" OR "Classical Swine Fever" OR "Foot and Mouth Disease" OR "Avian Influenza" OR "H5N1" OR "Newcastle Disease") AND TS=(virus OR viral) NOT TS=(vaccin* OR pathogenesis OR hemagglutinin OR haemagglutinin OR pathway

OR immun* OR interferon OR cytotox* OR lymphocyt* OR macrophag* OR antigen OR virulence OR antibod* OR phage OR "amino acid" OR "nucleic acid" OR antibiotic OR neuraminidase OR inhibitor OR "plant extract" OR intravenous OR embryo OR "human infection" OR recombinant OR clinical OR loop-mediated OR "rapid detection" OR peptide OR gene) NOT TI=(outbreak))
AND LANGUAGE: (English)
Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Timespan=All years

Search result: 349 hits

380+349= 729 in total, of which 191 duplicates > 538 articles resulting after removal of the duplicates

After screening these 538 articles based on the title, 97 (18%) were considered potentially relevant. 52 of those articles, mentioned in the section References were used to obtain relevant information for this study.

2.2 Criteria for including literature data in the database

Data obtained should meet the following criteria for inclusion in the database:

- Temperatures tested should be above 45°C
- Inactivation reached should be >0.5 log.
- At least two time points have to be reported and they have to be above detection limit.

From the articles providing quantitative inactivation data D values were retrieved. D value is the time (in minutes) required for 1 log inactivation of the virus (reduction by 90%). These data-points (log D) were plotted against the treatment temperatures. A z value (temperature change in °C required to change the D value by a factor of 10=1 log) can be obtained from the slope of the linear regression curve of the above-mentioned plots.

2.3 Additional references from the APHA report for FMD and PRRS

Additionally, for foot and mouth disease the references in the APHA risk assessment (Adkin et al. 2014) were analysed and results added into the database if the data was applicable. This was done for the following four references (Pharo 2002, Ryan et al. 2008, Donaldson 2011, Williams 2017). One additional reference was included for the PRRS virus (Bloemraad et al. 1994)

2.4 Articles not evaluated

Of the 97 articles considered potentially relevant, 31 articles were not included in the database because of constraints of the available time for this project. References to these 31 articles are included below and in the References list in the end of this report. These are mostly about heat resistance of foot and mouth disease virus reported in the years 1959 -1999. For this virus there is already considerable data included in the database which allows to predict global inactivation parameters.

These articles are also included in a subfolder in the Endnote library file.

(Dimopoullos et al. 1959, Bachrach et al. 1960, Fellowes 1962, Hiatt 1964, Forbes and Cottral 1969, Sellers 1969, Digioia et al. 1970, Parker 1971, Gough 1973, Callis et al. 1975, Hyde et al. 1975, Blackwell and Hyde 1976, Cunliffe et al. 1979, Deleeuw et al. 1980, McKercher et al. 1980, Doel and Baccarini 1981, Blackwell et al. 1982, Nettleton et al. 1982, Terry et al. 1983, Blackwell 1984, Böhm 1984, Blackwell and Rickansrud 1989, Vermeulen et al. 1993, Masana et al. 1995, Masana et al. 1995, McColl et al. 1995, Pagliaro et al. 1996, Ajariyakhajorn et al. 1997, Dekker 1998, Turner et al. 1998, Turner et al. 1999).

3 Results

3.1 Heat Resistance of viruses

Considerable amount of data for heat inactivation parameters was collected for the viruses relevant for this study. For AI, FMD, ND, and PRRS the number of data retrieved from 7, 10, 6 and 2 articles respectively was used to predict global inactivation parameters (Figures 2-5). Even though the data for PRRS was limited, modelling was done in view of its importance as a highly heat resistant virus. For PED and ASF two articles yielded inactivation parameters which are presented in a graph (Figures 6, 7), however this data is not enough to reliably make predictions. For CSF there was one article used to obtain data, which is presented in the Figure 8. The data is collected in the provided Excel files.

The water content of the product was an important factor in heat inactivation of viruses. This can be seen from Figure 2 (AI) and Figure 4 (ND) where for both AI and ND viruses the heat resistance measured in dried egg white as a substrate were relatively high, clearly outlying the other data-points which were mostly for liquid products (Fig 2 and 4). Besides the water content, also a high fat content (Bidawid et al., 2000) and the presence of clumping proteins, such as in chicken meat homogenate (Alexander and Manvell 2004) can protect viruses from heat inactivation.

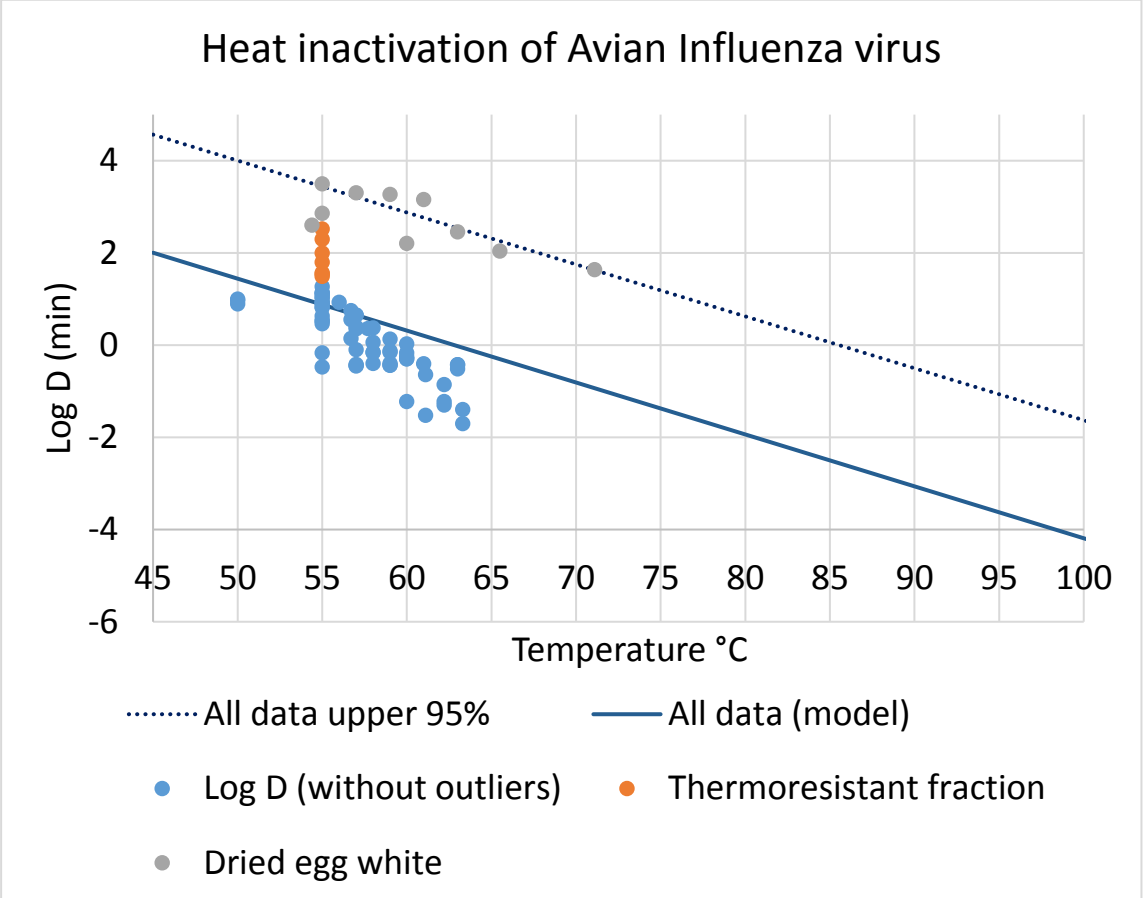

Figure 2. Heat inactivation of AI virus (Swayne and Beck 2004, Isbarn et al. 2007, Thomas et al. 2008, Thomas and Swayne 2009, Negovetich and Webster 2010, Chmielewski et al. 2011, Chmielewski et al. 2013). Heat inactivation data obtained from literature in the form of Log D values is plotted against the temperature. Linear regression is used to estimate the global inactivation parameters (z value) and is presented as a solid line. The dotted line is the upper 95% prediction interval (PI), which means that a new observation has a 95% chance of being within these limits.

Interpretation: investigating this graph at 70°C shows that on average logD=-0.81, so D=0.155 minutes (10-0.81), and a 30 minutes process would give 194 log reduction (30/0.155). This can be regarded as full inactivation.

The 95% upper line gives a D value of 56 minutes and a reduction of only 0.5 logs, so virtually no inactivation.

The depicted thermostable fraction in the graph is based on the inactivation rates for a virus population presumably consisting of two fractions: thermosensitive and thermostable (Negovetich and Webster 2010), which can be described by a biphasic type of inactivation curve. The inactivation rate for the sensitive fraction was approximately 14 times higher than that for the stable fraction (Negovetich and Webster 2010).

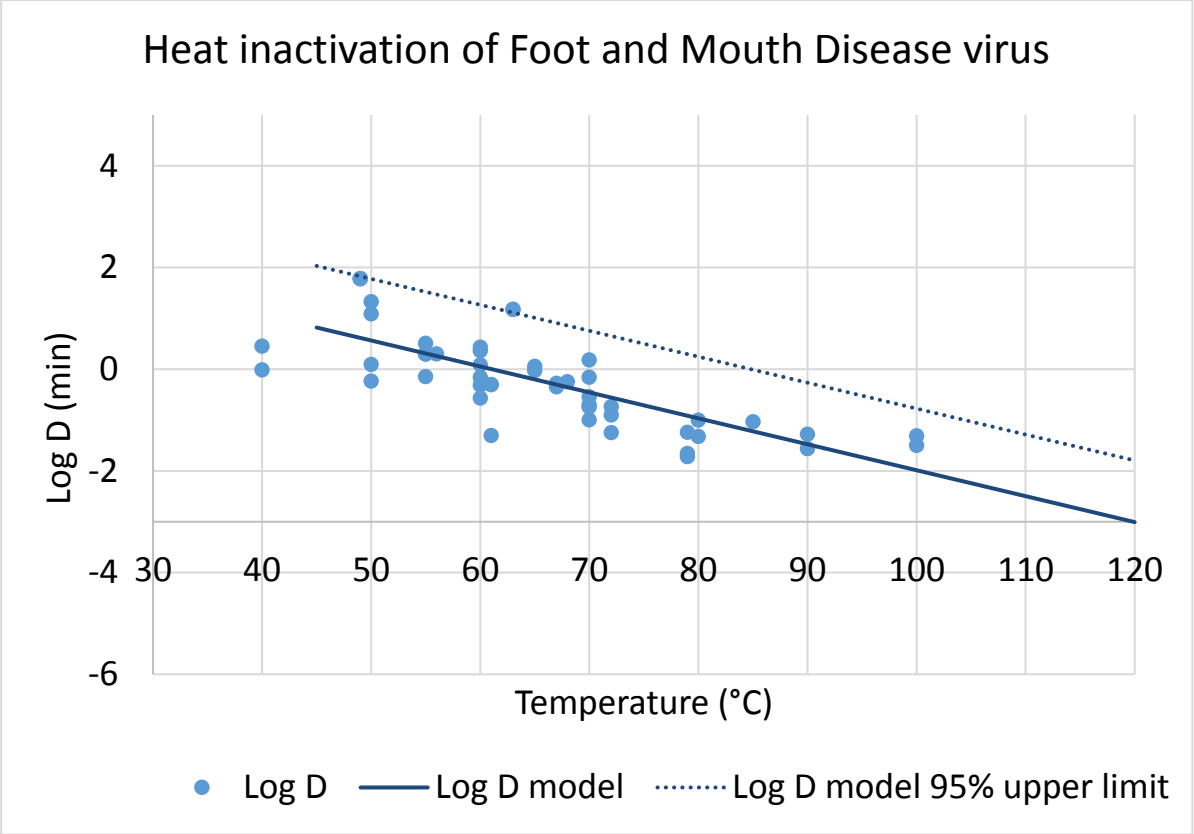

Figure 3. Heat inactivation of FMD virus (Bachrach et al. 1957, Bachrach 1959, Turner et al. 2000, Pharo 2002, Aly and Gaber 2007, Kamolsiripichaiporn et al. 2007, Ryan et al. 2008, Donaldson 2011, Gubbins et al. 2016, Williams 2017). Heat inactivation data obtained from literature in the form of Log D values is plotted against the temperature. Linear regression is used to estimate the global inactivation parameters (z value) and is presented as a solid line. The dotted line is the upper 95% prediction interval (PI), which means that a new observation has a 95% chance of being within these limits. The data obtained for this virus does not contain outliers. All data presented in this graph was reported for liquid products or meat slurry, no dry products were included.

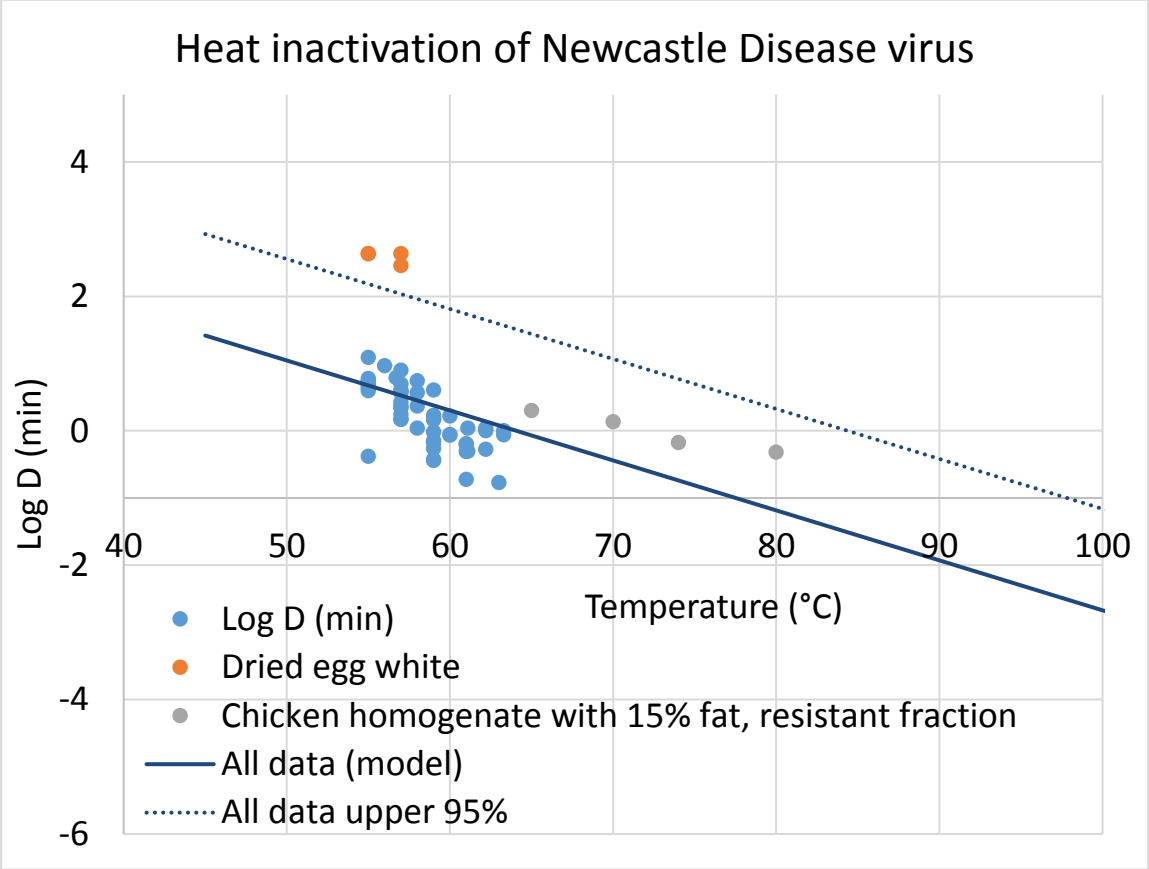

Figure 4. Heat inactivation of ND virus (Alexander and Manvell 2004, Swayne and Beck 2004, Thomas et al. 2008, Chmielewski et al. 2011, Chmielewski et al. 2013). Heat inactivation data obtained from literature in the form of Log D values is plotted against the temperature. Linear regression is used to estimate the global inactivation parameters (z value) and is presented as a solid line. The dotted line is the upper 95% prediction interval (PI), which means that a new observation has a 95% chance of being within these limits.

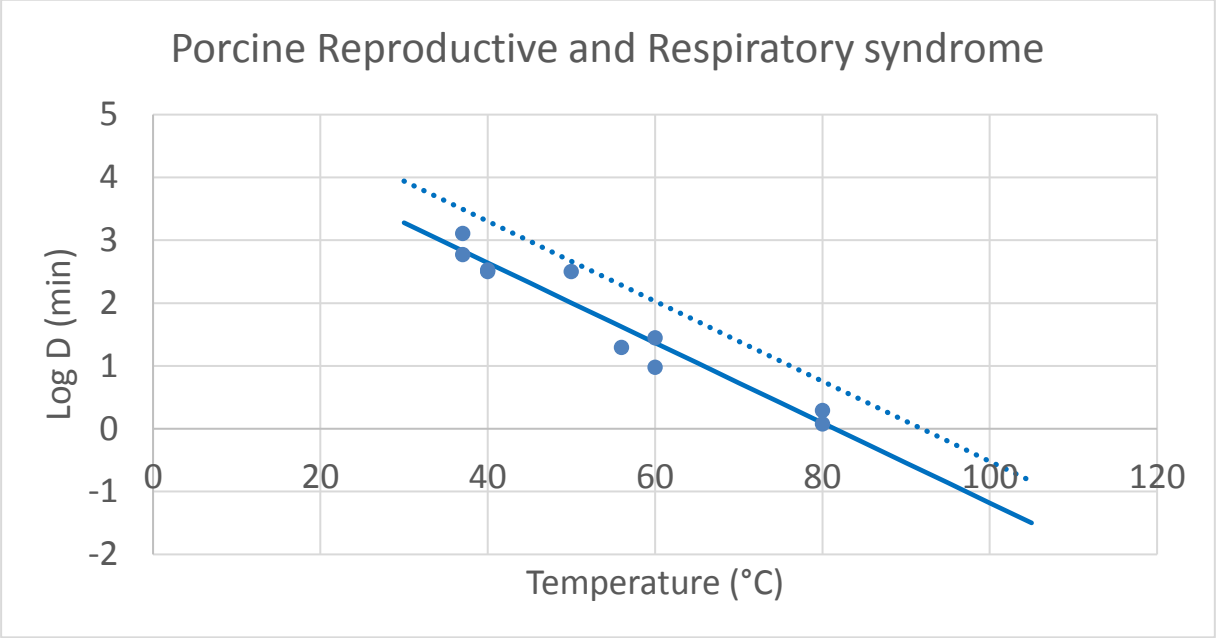

Figure 5. Heat inactivation of PRRS virus (Bloemraad et al. 1994, Linhares et al. 2012). Even though the number of obtained data-points was limited, estimates were obtained.

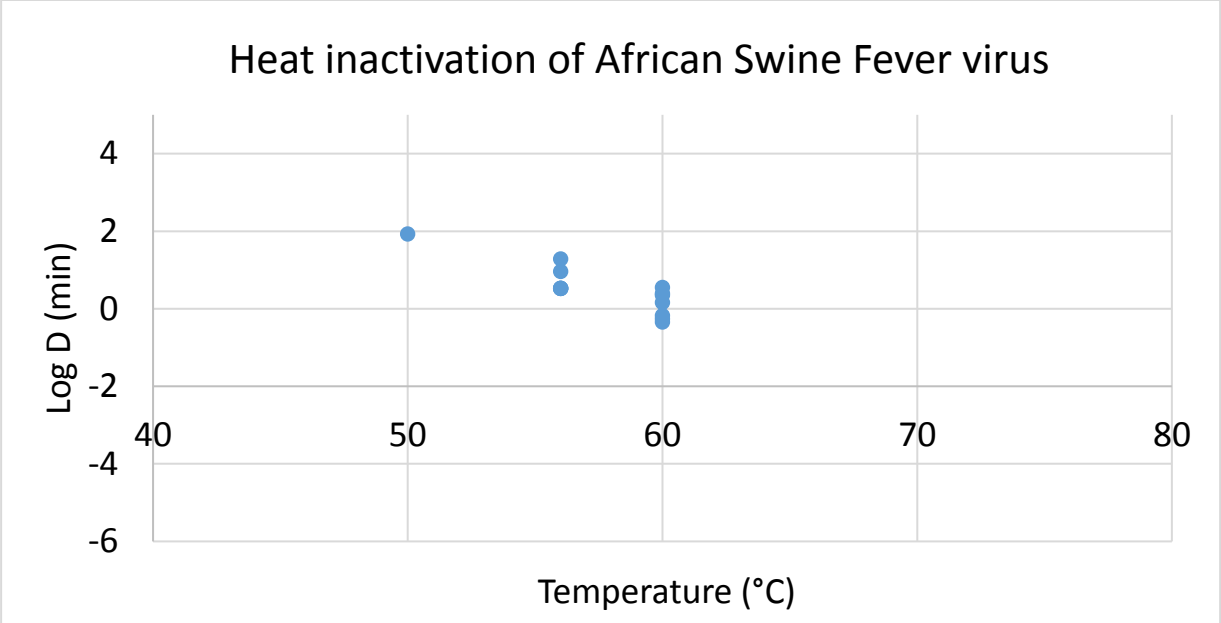

Figure 6. Heat inactivation of ASF virus (Plowright and Parker 1967, Turner and Williams 1999). The number of obtained data-points was not enough to obtain reliable predictions, therefore no modelling was performed.

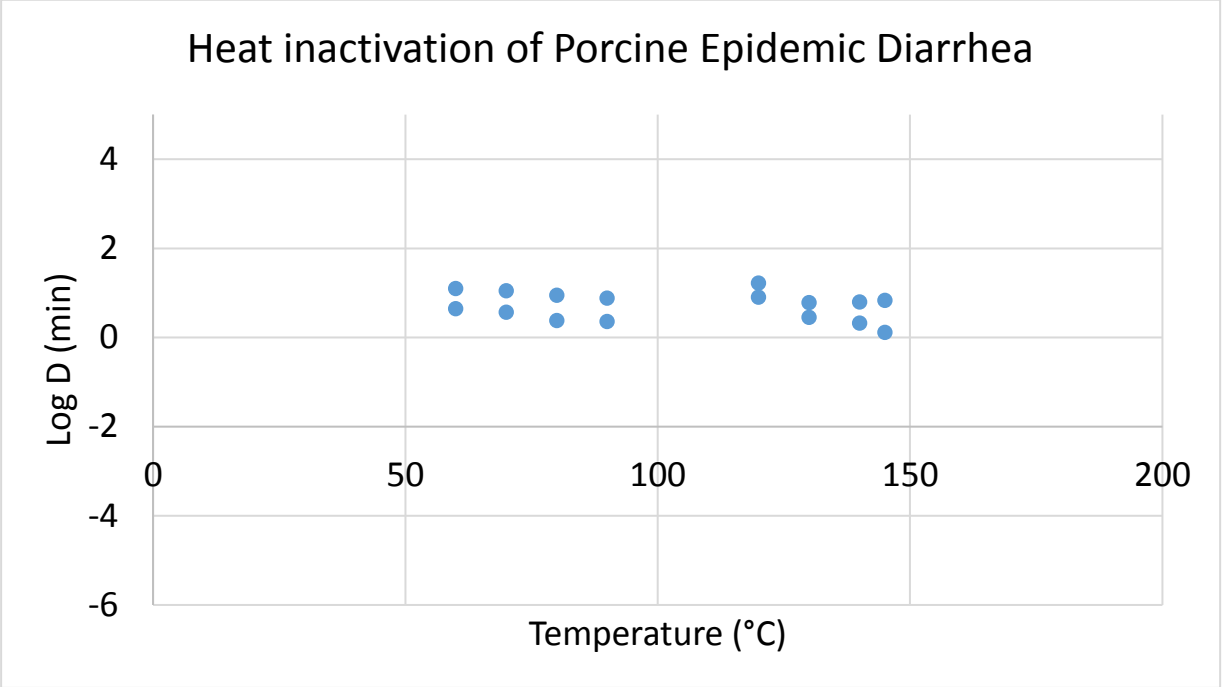

Figure 7. Heat inactivation of PED virus (Trudeau et al. 2016, Trudeau et al. 2017). The number of obtained data-points was not enough to obtain reliable predictions therefore no modelling was performed.

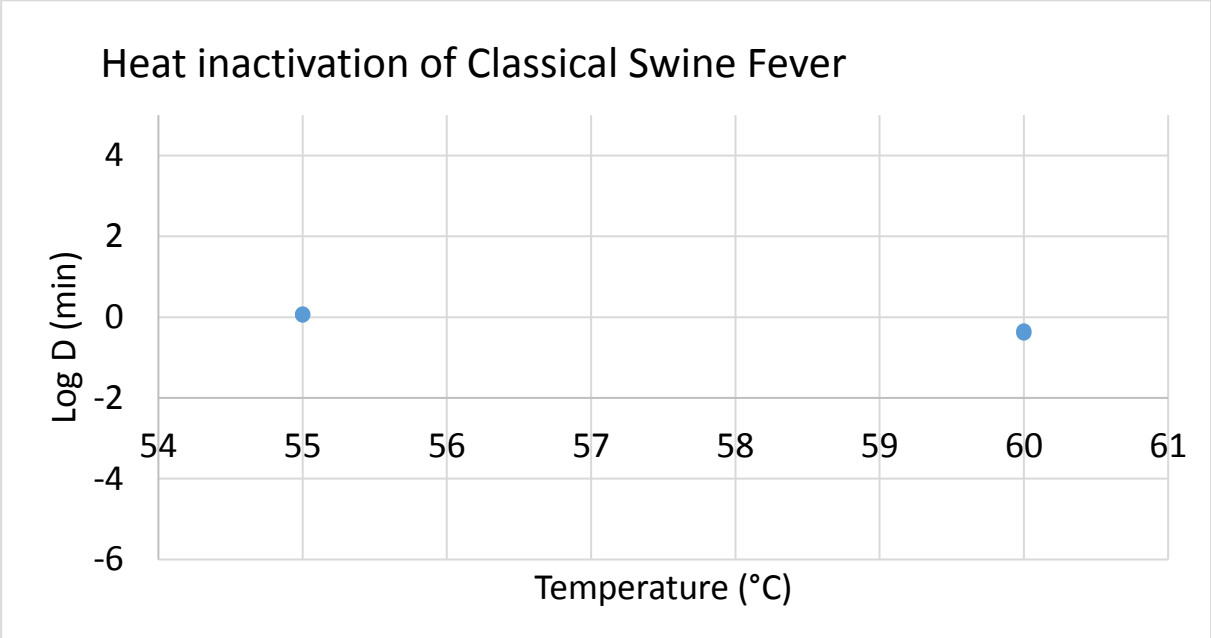

Figure 8. Heat inactivation of CSF virus (Turner et al. 2000). The number of obtained data-points was not enough to obtain reliable predictions. Therefore no modelling was performed.

Based on the models presented in Figures 2-4 it is possible to calculate log reductions for different heat/time scenarios using the estimated z value and the log reduction at a chosen reference temperature (Tref). It is advised to use the upper 95% prediction interval instead of the average for the latter parameter for

more conservative estimates. Example scenarios along with estimated z-values and Dref are presented in Table 2. Similar calculations for other scenarios can be done using the Equation 1 or the provided Excel file. However note that the further the scenario is from the actual experimental data, the less certain will be the prediction. Furthermore, the predictions are highly dependent on the data available, for example for AI and ND virus there was data available for their heat inactivation in dried egg-white, and for FMD the data-points were only for liquid products and meat-slurry, which influences the outcome of predictions.

$$\text{Log } D = \text{Log Dref} - (T - Tref)/z \qquad \text{(Equation 1)}$$

Where:

log D is the logarithm of the D-value (log min)

log Dref is the log D-value at Tref (log min),

Tref is the reference temperature (°C), in this study equal to 70°C.

z is the temperature increase (°C) needed to reduce the D-value with a factor of 10.

Table 2. Estimated Log reductions after 30 min of heating at specified temperatures for AI, FMD, ND and PRRS based on the model prediction upper 95% limit. Estimated z-values and D_{ref} are also presented.

Virus	T_{ref} (°C)	D_{ref} (min) (Upper 95% prediction limit)	z value (°C)	Estimated Log reductions in 30 min (Upper 95% prediction limit)	
				At T_{ref}=70°C	At 100°C
Avian Influenza (all products, including dried egg-white)	70	56	9	0.5	1275
Foot and Mouth Disease (liquid products)	70	5.7	20	5	179
Newcastle Disease (all products, including dried egg-white)	70	11.7	13	2.6	436
Porcine Reproductive and Respiratory syndrome (PRRS) (liquid products)	70	25	15.7	1.2	99

3.2 Observations from the literature regarding heat resistance

3.2.1 General observations

Below several points are listed that were reported in literature and that are relevant (but not in the scope of the analysis in this study).

- Several studies report that if the virus was no longer detected in the sample by culture methods, the sample could still be infective for animals. For example, after 36 seconds heating at 82.5 °C of milk samples containing initially 2 logs/ml for FMD, the virus could not be detected by the applied culture method, however the milk was still able to cause infection in a steer (Tomasula et al. 2007). Increasing the pasteurisation conditions of the milk to 95°C for 36 s, still resulted in infection of the steer.
- Some food components, such as fat, solid products (Ryan et al. 2008), and salt (1%) (Blackwell et al. 1982) may have a protective effect on the virus during the heat inactivation
- In many studies both for heat resistance and for acid resistance especially in case of not very severe treatments a fraction of the virus may survive the treatment (~1/1'000'000), the so called tailing effect. Some studies provided also inactivation data for this fraction, which was included in the database and is referred to as thermo-resistant fraction (Fig. 2 and 4) (Plowright and Parker 1967, Alexander and Manvell 2004, Negovetich and Webster 2010).

3.2.2 Foot and mouth disease: Thermal death time curve in milk from literature

Figure 1 ((Tomasula and Konstance 2004), reprinted from Walker 1984) illustrates the thermal death curve of the FMD virus in milk. This is based on experimental data, and is an alternative approach to modelling based on D values (time for 90% reduction), since only complete inactivation or survival type of data is included, irrespective of initial concentrations. It can be seen for example that heating at 100°C for 27 minutes (1620 s) should be enough to inactivate the FMD virus in milk. The limitation of this kind of data is that the degree of inactivation (for example D value) is not well defined and complete inactivation is assumed based on the detection limit of the experiment.

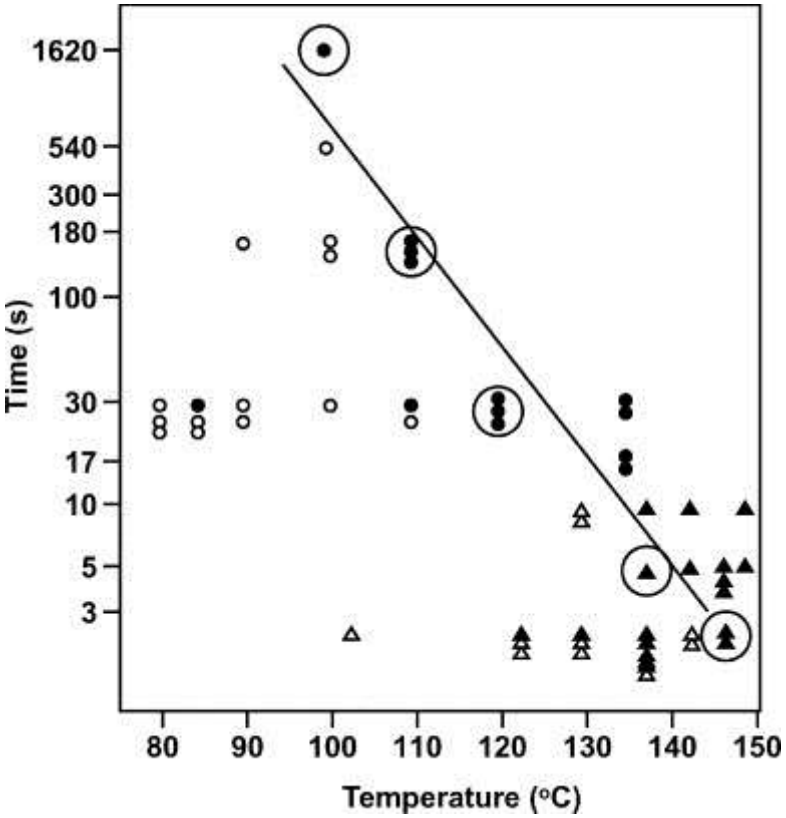

Figure 1. Thermal death time curve for Foot-and-Mouth Disease virus (FMDV) in skim or whole milk from infected cows. Data points obtained at the Central Veterinary Institute, Lelystad, The Netherlands. • = FMDV inactivated; ○ = FMDV survived. Data points obtained at Plum Island Animal Disease Center (Greenport, NY): ▲ = FMDV inactivated; △ = FMDV survived. Points with large open circles were used to hand-fit the data. Reprinted from Walker et al., "The thermal death time curve for foot-and-mouth disease virus contained in primarily infected milk." 1984. *BIOLOGICALS* (formerly *J. Biological Standardization*) 12:185–189 with permission from Elsevier.

3.3 Resistance of viruses to acid (low pH) or fermentation process

Database

A limited amount of studies providing quantitative data for acid/pH inactivation of viruses was retrieved. No modelling was performed but the retrieved data from literature in the form of D-values is presented in the graphs in Figures 9-15, for AI, ASF, PRRS, FMD, CSF, PED and ND viruses.

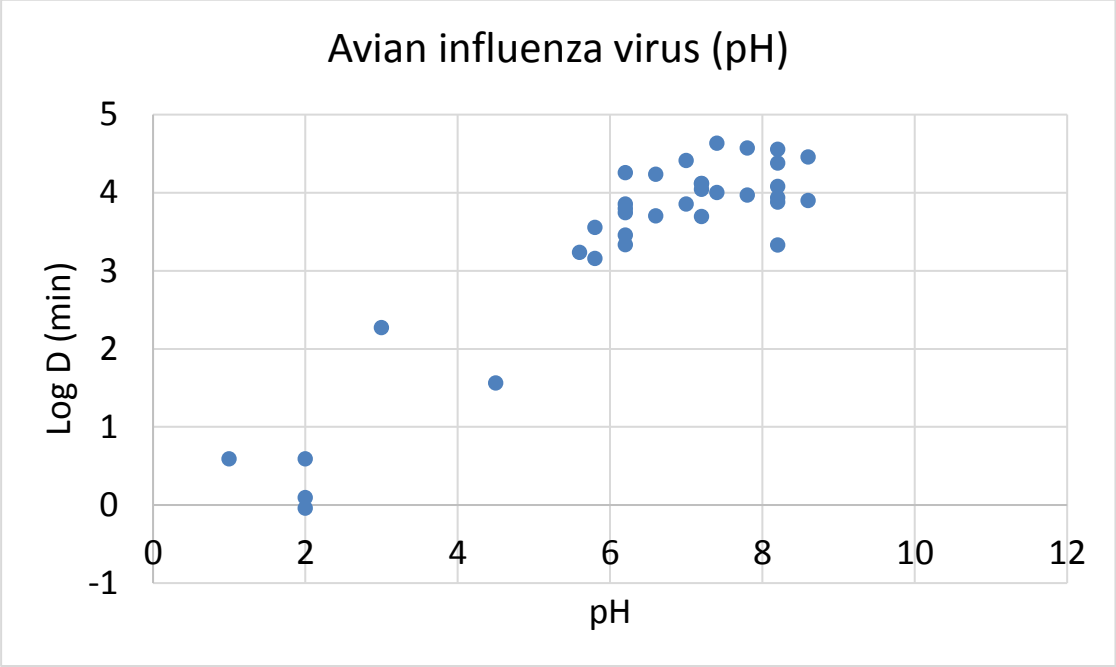

Figure 9. Sensitivity of AI virus to pH (17-28°C) (Stallknecht et al. 1990, Lu et al. 2003, Brown et al. 2009, Kabell et al. 2009, Vinneras et al. 2012, Zou et al. 2013, Lange-Starke et al. 2014).

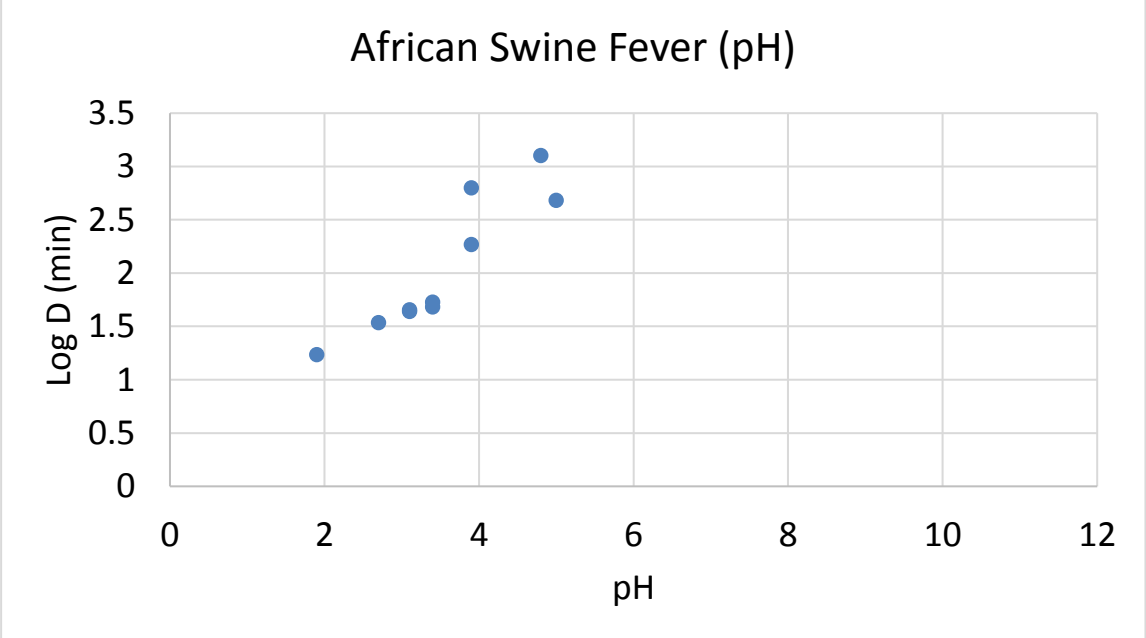

Figure 10. Sensitivity of ASF virus to pH (18°C) (Plowright and Parker 1967).

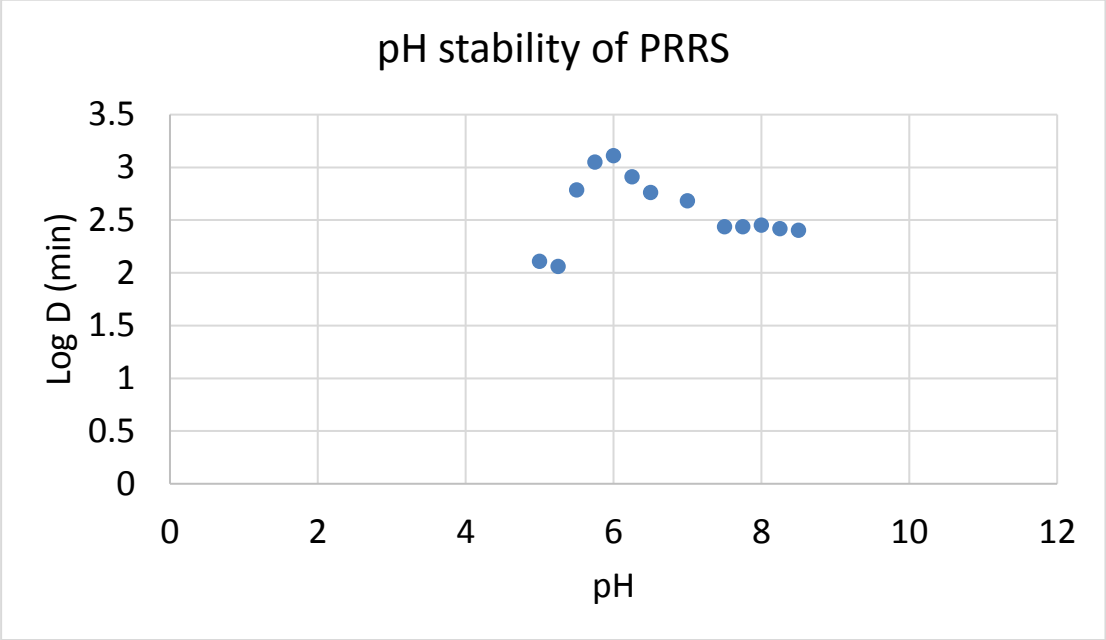

Figure 11. Sensitivity of PRRS virus to pH (37°C) (Bloemraad et al. 1994).

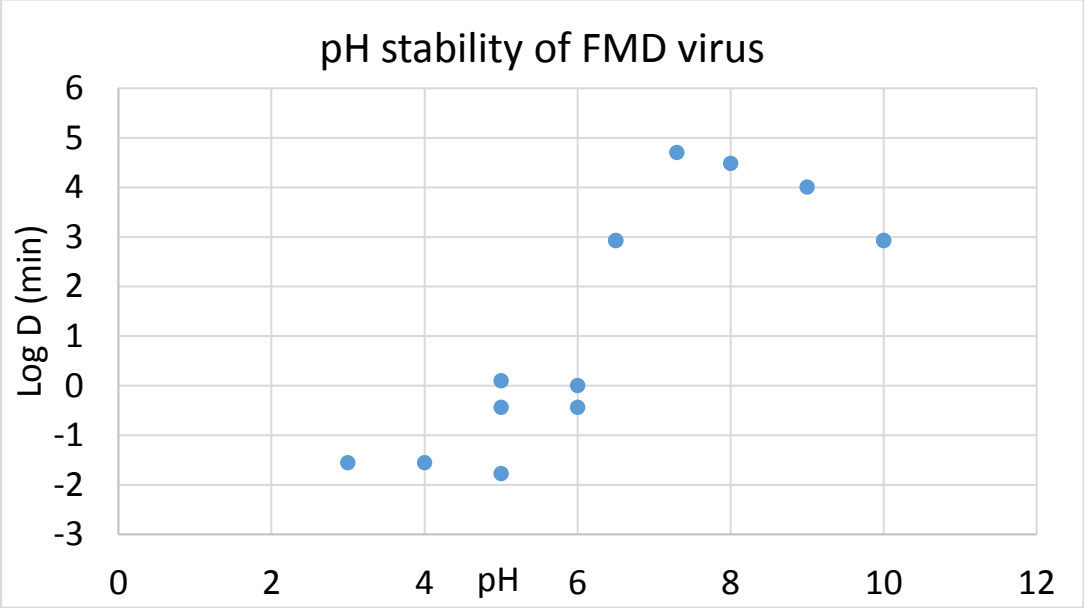

Figure 12. pH stability of FMD virus (4°C) (Bachrach et al. 1957, Bachrach 1959, Pharo 2002). Testing was done in isotonic solution where, according to the author FMD virus appears to be unique in its sensitivity to very slight acidification (Bachrach 1968).

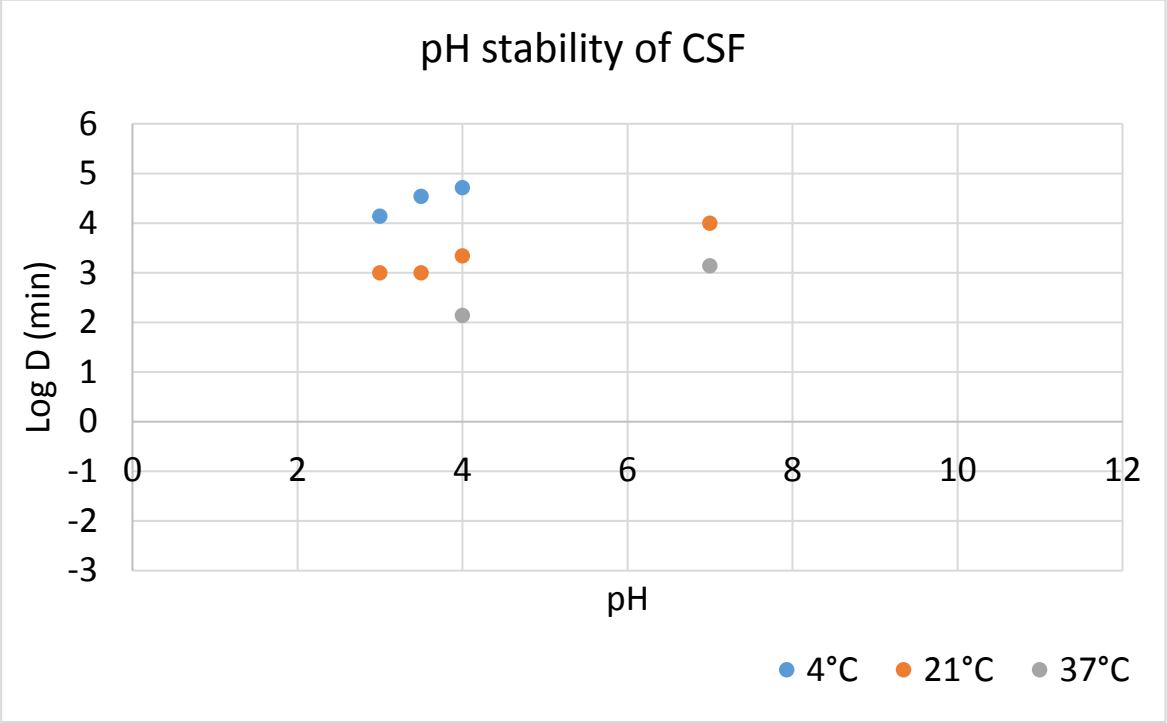

Figure 13. pH stability of CSF virus (4-37°C) (Edwards 2000).

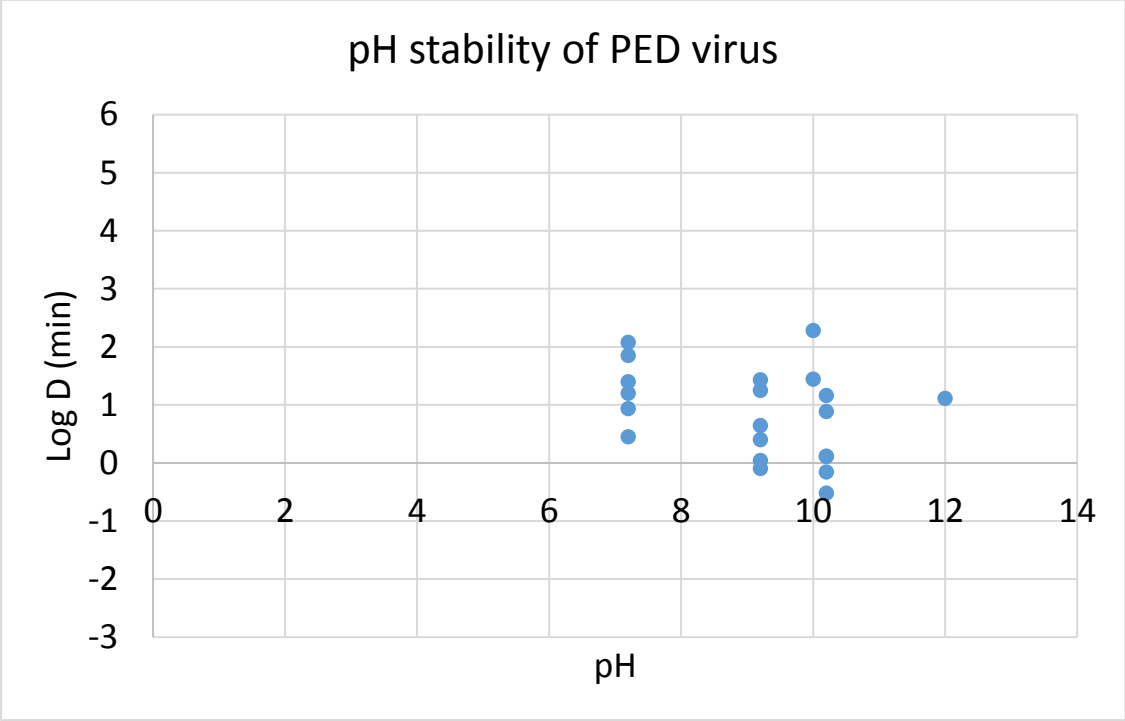

Figure 14. pH stability of PED virus (at 40-48 °C, effect of alkalyzation) (Quist-Rybachuk et al. 2015, Stevens et al. 2018).

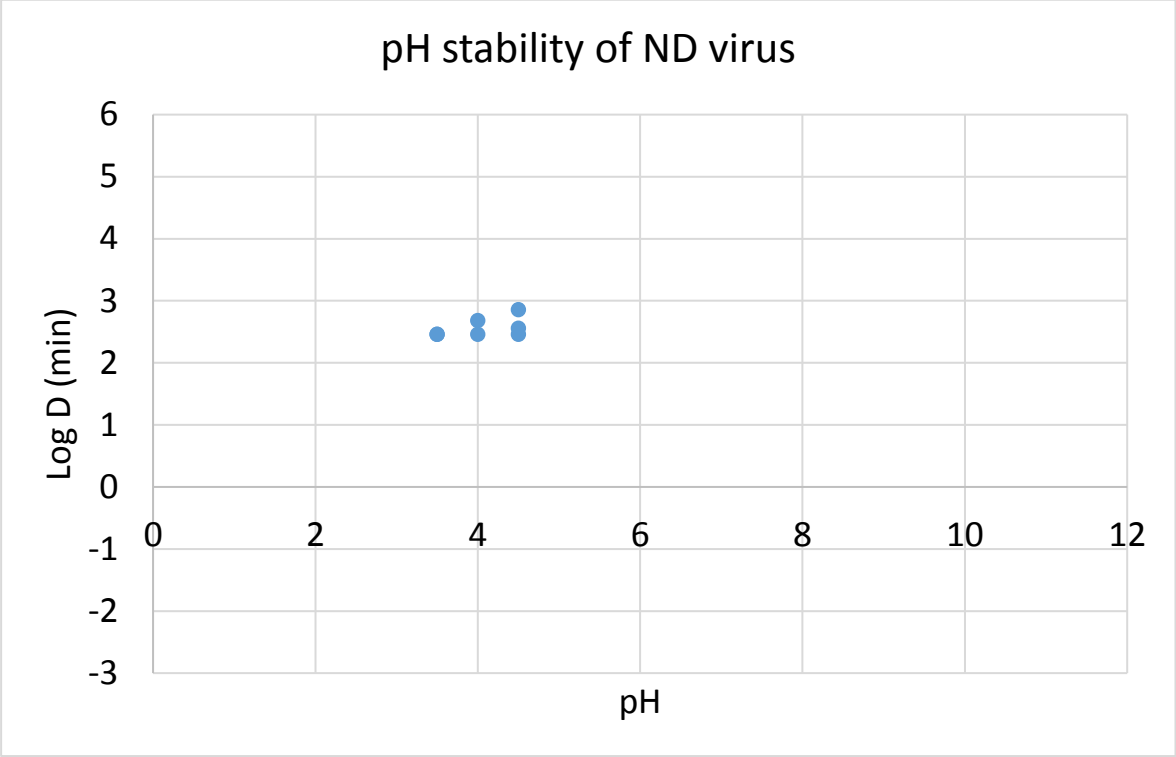

Figure 15. pH stability of ND virus (at 20-40 °C) (Gilbert et al. 1983).

3.4 Information from literature regarding resistance to acid/pH/fermentation

The section below describes information retrieved from articles that did not meet criteria for inclusion in the database however was still relevant. This information describing the resistance of several viruses to low pH/acidification or fermentation obtained from literature is presented below.

3.4.1 ND virus

Survival during fermentation

ND virus was able to survive the fermentation process of edible waste material for up to 96 hours at all temperatures tested (Wooley et al. 1981). The pH drop in this study was dependent on the temperature. At 5°C the pH drop was less than by 0.5 units, at 10°C it was decreased by 0.5 (from 4.7-4.2=0.5). The drop was more significant at higher temperatures, at 20°C it was from pH=4.7 to 3.6, and at 30°C it was from pH=4.7 to 3.3 after 96 hours.

In another study observations were carried out for a longer time period of up to 8 days of fermentation with Lactobacillus acidophilus or Saccharomyces cerevisiae. Results show that the **ND** virus was inactivated after 7 days at 20°C and day 6 at 30°C when fermented with S. cerevisiae. In case of the Lactobacillus fermentation, the virus survived on solid samples for 7 days at 20°C, 2 days at 30°C, and less than 24 h at 40°C. In the liquid samples, NDV survived 6 days at 20°C and between 24 and 48 h at 30 and 40°C. In this study the observed pH drop during 8 days was also temperature dependent, at 20°C from pH=4.5 to 3.5 (L. acidophilus) or pH=5 to 3.7 (S. cerevisiae), and at 30 and 40°C from pH=4.5 to 3 (L. acidophilus) or pH=5 to 3.3 at 30°C (S. cerevisiae) (Gilbert et al. 1983). In this study the pH sensitivity of the virus was tested in a culture medium and at pH=3.5 at 20C there were still survivors up to day 9, whereas at 40C at pH=3.5 and 4 there was no virus detected already after 1 day.

In another study for waste fermentation **ND** virus survived 4 days at 20 °C, 2 days at 30 °C, and 1 day at 40 °C (Shotts Jr et al. 1984). The maximum pH drop in this study during 9 days was from pH=5.5 to 3.5 at 30 and 40°C, and to pH=4 at 20°C.

pH effect

ND was not inactivated with ascorbic acid when applied at concentrations up to 0.2 mg/ml and at neutralised pH 7.4-7.6 (Sinha and Datta 1950). Several strains of ND survived between pH=4-11 from 1 hour to up to 1 day. These viruses lost their infectivity only at pH=12 and <3 after 1 hour (Moses et al. 1947). At pH 4 some of the viruses still did not lose their infectivity after 24 hours, however storage of 7 days at pH=4 lead to loss of their infective capacity (Tolba and Eskarous 1959).

3.4.2 FMD virus

pH effect

The FMD virus is more sensitive to low pHs and was completely inactivated within a few seconds at pH values of below 4 (Bachrach et al. 1957). This virus was rapidly inactivated at pH=3.4 and below reaching about 5 log reduction in 4 hours, however there was a small resistant fraction remaining active even after 20 hours (Plowright and Parker 1967). The resistant fraction (1 millionth part) remaining active was also observed in other studies (Bachrach et al. 1957). pH=5 reduced the virus numbers by 4 logs in 5 min (Bachrach 1959). FMD virus (5 logs) in milk was completely inactivated after the production of yoghurt (pH 4.3) by using the contaminated milk, and was not further detected during 2 d of refrigerated storage (pH 4.0) (Aly and Gaber 2007).

FMD virus is most stable at pH=7 to 7.5. Further away the pH value shifts in both directions, the faster the virus is inactivated. For example at pH 6 and 10 there was a 90% reduction in infectivity every 14 hours (D=14 hours), whereas at pH=5 and 6 similar reduction was observed in less than 1 min. The inactivation of the virus at pH=2, 3 and 4 was very rapid (Bachrach et al. 1957).

Survival in cheese matrix

FMD virus was able to survive the cheese making process of Cheddar and Camembert cheeses, though not that of Mozzarella. The virus survived the processing but not curing for 30 days in Cheddar cheese prepared from heated milk. However, the virus survived curing for 60 days but not for 120 days in cheese (pH 5) prepared from unheated milk. Foot-and-mouth disease virus survived in Camembert cheese (pH 5) for 21 days at 2 °C but not for 35 days (Blackwell 1976). The pH reached during the cheese making process for the above mentioned cheeses is not low enough to provide quick inactivation of the virus.

Acidification with consumable acids

Acidification with 0.1%-0.3% of consumable acids, such as citric acid or propionic acid, could not guarantee the complete inactivation of **FMD** virus in skimmed milk (Sonder et al. 1990). This was due to flocculation of milk proteins which provided protection to the virus at low pH (~<5). And at pH>5.5 the acidification was not enough to provide reliable virus inactivation. Thus there was a narrow pH range where the complete inactivation of the virus was observed after 6 hours, for example pH=5.16-5.60 for acidification with propionic acid (0.1-0.3%) or pH=4.62-5.76 with citric acid (0.1-0.3%).

3.4.3 PEDV virus

PEDV lost infectivity after being exposed to pH<4 or pH>9 at 37°C for 6 hours (Hofmann and Wyler 1989).

3.4.4 ASF virus

For the **ASF** virus, similarly, the critical pH value below which the virus is relatively quickly inactivated was pH=3.4. However, a small resistant fraction remained inactivated for up to 21 hours. Below pH=2.7 there was no more resistant fraction observed (Plowright and Parker 1967).

4 Concluding remarks

4.1 Heat resistance

A significant amount of data for heat inactivation was found in the scientific literature for three out of seven viruses considered in this study. Global inactivation parameters were estimated for those three viruses: AI, FMD and ND. These global inactivation parameters can be used to make global predictions about the viral inactivation for different heating scenarios.

The data show that the matrix has a large impact on the heat inactivation of the viruses. Dried products – exemplified in this study for dried egg white - results in higher heat resistance compared to liquid products.

An important point to be made is that when heat treatments are not severe enough, a subpopulation of the viral population may be distinguished which has higher resistance, referred to as resistant fraction. This information was included in the database whenever available. Therefore it is advised to consider the upper 95% intervals for predicted parameters.

For the other four viruses, PED, ASF and CSF, the amount of data was too limited to make reliable predictions, however this data is also presented in the graphs and can still be helpful in making comparisons.

An important point to take into account for application of heat treatment of viruses is also the initial contamination level and the desired level of inactivation. The desired level of inactivation is dependent on the severity of the outcome if the disease would occur and the presence of other control measures. For example, for bacterial contaminants, *Listeria monocytogenes* in pasteurised milk and *Escherichia coli* in orange juice 5 or 6 log reduction during the heat treatment is the target treatment, since there is no significant growth expected during the storage time. For a toxin producing pathogen *Clostridium botulinum* in canned foods sterilisation aims for a 12 log reduction due to the severity of the disease and the absence of other control measures preventing its growth. An even stricter control would be desired to prevent the introduction of a severely infective disease in a country. This is the case for many of the diseases considered in the risk assessment by APHA.

4.2 pH sensitivity/fermentation

Articles providing quantitative data for virus inactivation by pH and fermentation are limited. For AI and ASF viruses this data is provided in the graphs (without modelling). For other viruses this information from literature is summarised in the results section.

For virus inactivation during fermentation the pH drop and the temperature at which the substrate is fermented are important factors. This two factors are also interconnected since the pH drop is occurring faster at ambient temperatures of 20-40°C compared to chilled storage fermentation at <20°C. However the optimal temperature for waste fermentation needs to be selected cautiously taking into account not only the virus inactivation but also the growth of possible bacterial contaminants.

Several studies report long survival of viruses during fermentation. For example, ND virus was able to survive several days of fermentation, up to 6 days, even when the pH dropped to 3.6. Storage of up to 7 days at pH=4 was required in another study for ND to lose its infectivity. FMD was more sensitive to low pH and was inactivated during yoghurt production process (pH=4) within less than two days.

The viruses are most stable at neutral conditions at around pH=7. Further away the pH value shifts in both directions, the faster the virus is inactivated. PEDV lost infectivity after being exposed to pH<4 or pH>9 at 37°C for 6 hours. For the ASF virus, similarly, the critical pH value below which the virus is relatively quickly inactivated was pH=3.4. Some studies report on the protective effect of the presence of flocculating proteins, such as in milk, on acid inactivation of viruses.

For acid resistance various studies report evolvement of a small resistant fraction of the virus which may require longer time or more severe conditions of inactivation at milder pH values about. Similar findings were reported for heat resistance and mentioned above.

5 References

52 references which contained relevant information for this study.

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