General enquiries on this form should be made to:
Defra, Procurements and Contracts Division (Science R&D Team)
Telephone No. 0207 238 5734
E-mail: research.competitions@defra.gsi.gov.uk

SID 5 Research Project Final Report

- **Note**
  In line with the Freedom of Information Act 2000, Defra aims to place the results of its completed research projects in the public domain wherever possible. The SID 5 (Research Project Final Report) is designed to capture the information on the results and outputs of Defra-funded research in a format that is easily publishable through the Defra website. A SID 5 must be completed for all projects.
- This form is in Word format and the boxes may be expanded or reduced, as appropriate.
- **ACCESS TO INFORMATION**
  The information collected on this form will be stored electronically and may be sent to any part of Defra, or to individual researchers or organisations outside Defra for the purposes of reviewing the project. Defra may also disclose the information to any outside organisation acting as an agent authorised by Defra to process final research reports on its behalf. Defra intends to publish this form on its website, unless there are strong reasons not to, which fully comply with exemptions under the Environmental Information Regulations or the Freedom of Information Act 2000.
  Defra may be required to release information, including personal data and commercial information, on request under the Environmental Information Regulations or the Freedom of Information Act 2000. However, Defra will not permit any unwarranted breach of confidentiality or act in contravention of its obligations under the Data Protection Act 1998. Defra or its appointed agents may use the name, address or other details on your form to contact you in connection with occasional customer research aimed at improving the processes through which Defra works with its contractors.

Project identification

1. Defra Project code: SE4401

2. Project title:
   Assess if Thermo Resistant Viruses, such as Parvovirus, are a relevant hazard in Category 3 ABPs used as incoming materials in Biogas and Composting Plants.

3. Contractor organisation(s):
   Veterinary Laboratories Agency
   Woodham Lane
   New Haw
   Addlestone
   Surrey
   KT15 3NB

4. Total Defra project costs (agreed fixed price): £ 45,534

5. Project: start date 01.09.2010
   end date 31.03.2011

6. It is Defra's intention to publish this form.
Please confirm your agreement to do so..... **YES X** ☐ **NO** ☐

(a) When preparing SID 5s contractors should bear in mind that Defra intends that they be made public. They should be written in a clear and concise manner and represent a full account of the research project which someone not closely associated with the project can follow.
Defra recognises that in a small minority of cases there may be information, such as intellectual property or commercially confidential data, used in or generated by the research project, which should not be disclosed. In these cases, such information should be detailed in a separate annex (not to be published) so that the SID 5 can be placed in the public domain. Where it is impossible to complete the Final Report without including references to any sensitive or confidential data, the information should be included and section (b) completed. NB: only in exceptional circumstances will Defra expect contractors to give a "No" answer.
In all cases, reasons for withholding information must be fully in line with exemptions under the Environmental Information Regulations or the Freedom of Information Act 2000.

(b) If you have answered NO, please explain why the Final report should not be released into public domain

Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

Commission Regulation (EC) 208/2006 and Regulation EC 1774/2002 dictate the conditions for composting Category 3 animal by-product (ABP) materials. Specifically, the Regulations specify a maximum particle size of 12 mm, and minimum temperature of 70°C for at least 60 minutes in a closed system. The competent authority may also authorise the use of other standardised processes including, for example, the use of composting or biogas by waste processing companies. Under EC 208/2006, the applicant is required to demonstrate that such parameters ensure that biological risks are minimised. The demonstration includes a validation of the intended process and in particular a 5 $\log_{10}$ reduction of *Salmonella senftenberg* (77W, H_2S negative) and at least a 3 $\log_{10}$ reduction in infectivity titre of thermo-resistant viruses such as parvovirus when they are identified as a relevant hazard.

The objectives of this project are:-

i) to identify those thermo-resistant viruses which may pose a relevant hazard in Category 3 ABPs used as raw materials in biogas and composting plants;
ii) to generate a thermo-stability plot for a 3-log reduction for each virus; and
iii) to compare thermo-stability plots for 3-log reductions of virus with a 5-log reduction of *S. senftenberg*.

After discussing with experts in VLA virology, a list of 20 viruses was selected for further study (Milestone 1) and thermo-stability plots were developed (Milestone 2). Those viruses were selected on the basis that they may be present in Category 3 ABP and are non-enveloped. The phospholipid bilayers of enveloped viruses disintegrate at high temperature making the virus more susceptible to heat than non-enveloped viruses. A literature search was conducted to obtain published and unpublished data on the thermo-stability of the 20 viruses selected from the hazard identification together with *S. senftenberg*.

The availability of the thermal inactivation data varied widely between the pathogens. Thus for some viruses (e.g. rabbit haemorrhagic disease virus and bluetongue virus) limited data covering only a few temperatures were available whilst for other viruses (e.g. foot-and-mouth disease virus and bovine parvovirus) data were more abundant and covered a range of temperatures. For two viruses, namely "other" circoviruses and avian parvoviruses, no data was available to construct thermo-stability plots. Therefore, plots were generated for 18 viruses and *S. senftenberg*.

From the literature review, it was ascertained that the log reduction with time was non-linear for the viruses studied and for *S. senftenberg*. Therefore, two thermo-stability plots were generated to take into account

all of the available information: one plot for a 1-log reduction and a second plot for a 3-log reduction. It was also noted during the literature review that there were several different media used in the experimental studies and these appeared to have an effect on the thermo-stability of the viruses and bacteria. To account for this, the media were broadly categorised into 1) aqueous (e.g. water, buffer or serum), 2) other (e.g. slurries and manures) and 3) meat.

The nature of the outputs from the published and unpublished studies was highly variable with some studies reporting a 1-log reduction while others reported up to a 5-log reduction in various media (e.g. *S. senftenberg*). To analyse the data in a consistent and transparent manner, several guidelines were agreed upon by the project team. The times required for a 3-log or 1-log inactivation at each temperature were plotted for each virus and a line of best fit was determined.

After plotting data for each of the viruses and *S. senftenberg* individually, a single plot was generated for a 3-log reduction in aqueous media to ascertain the relative ranking of the viruses' thermo-stabilities. Based on the caveats of the available data (i.e. variation in strains, media types, data availability), some overall conclusions can be drawn. These are:

- the nature of the medium has an impact on the thermo-stability of the viruses with, in general, thermo-stability of the viruses and *S. senftenberg* being greatest in "meat", "aqueous" and then "other" media.
- parvoviruses (bovine parvovirus (BPV) and porcine parvovirus (PCV)) appear to be the most resistant to heat at 56°C, 60°C and 70°C when considering a 3-log reduction and are clustered on the thermo-stability plots.
- at 70°C for an hour, all of the viruses studied, except for infectious pancreatic necrosis virus and parvo-viruses, appear to exhibit a 3-log reduction in infectivity, in aqueous media
- Given the data caveats and assumptions, *S. senftenberg* is not an appropriate marker for ensuring that a 3-log reduction has been achieved in viral hazards potentially present in Category 3 ABPs.
- Parvovirus, particularly bovine and porcine, is an appropriate marker for ensuring that a 3-log reduction has been achieved in viral hazards potentially present in Category 3 ABPs.
- The results, given the data caveats and assumptions, support the EC 208/2006 Regulations to use bovine parvovirus as a marker of viral inactivation.

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:
   - the scientific objectives as set out in the contract;
   - the extent to which the objectives set out in the contract have been met;
   - details of methods used and the results obtained, including statistical analysis (if appropriate);
   - a discussion of the results and their reliability;
   - the main implications of the findings;
   - possible future work; and
   - any action resulting from the research (e.g. IP, Knowledge Transfer).

1. Overview

Animal by-products (ABPs) include animal carcasses, parts of carcasses and products of animal origin. Examples of such products are: catering waste, butcher and slaughterhouse waste, blood, wool, hides and skins, fallen stock and manure. The disposal of ABPs is strictly regulated and depends upon the potential risk that the product poses to animals, the public and to the environment. This risk is determined by categorising ABPs into 3 classifications: Category 1, Category 2 and Category 3. Category 3 ABP comprises the lowest-risk materials and includes raw meat that has passed meat inspection but is not intended for human consumption, material such as feathers, eggshells, wool, and horns from animals that do not show any signs of disease and also waste from food manufacturers and retailers. Category 3 APBs can be disposed in various ways including incineration, rendering, composting and anaerobic digestion.

Commission Regulation (EC) 208/2006 and Regulation EC 1774/2002 (the 'EU Animal By-Products Regulation'), dictate the conditions for composting of Category 3 ABPs. Specifically, the regulations stipulate that Category 3 material to be used as raw material for biogas or composting must be treated at 70°C for 1 hour in a closed system with a particle diameter no greater than 12mm. The competent authority may authorise the use of other standardised processes including, for example, the use of composting or biogas by waste processing companies. Under EC 208/2006, the applicant is required to demonstrate that such parameters ensure the biological risks are minimised. The demonstration includes a validation of the intended process and in particular a 5 $\log_{10}$ reduction of *Salmonella senftenberg* (77W, H_2S negative) and at least a 3 $\log_{10}$ reduction in infectivity titre of thermo-resistant viruses such as parvovirus when they are identified as a relevant hazard. Given that it is uncertain whether the choice of parvovirus in the Regulations is risk based, a question arises as to whether parvovirus is a suitable marker for viral hazards and if it is the most thermo-resistant virus that may be present in Category 3 ABPs.

Depending on their origin, it is possible that Category 3 ABPs contain viruses of animal and public health significance (e.g. avian influenza virus, foot-and-mouth disease virus and blue tongue virus). It is well documented that some viruses are more thermo-resistant than others. For example, in general, enveloped viruses are less heat resistant than those that do not have a phospholipid membrane (non-enveloped viruses). Those viruses that are highly thermal resistant and are transmitted through meat and meat products may represent a relevant hazard.

The objectives of this project are:-

1. to identify those thermo-resistant viruses which may pose a relevant hazard in Category 3 ABPs used as raw materials in biogas and composting plants;
2. to generate a thermo-stability plot for a 3-log reduction for each virus; and
3. to compare thermo-stability plot for 3-log reductions of each virus with a 5-log reduction thermo-stability plot for *S. senftenberg* (775W, H_2S negative).

These objectives have been met through delivery of reports to Defra for Milestone 1 (hazard identification), Milestone 2 (thermo-stability plots) and Milestone 3 (final report and presentation). The presentation was given (22nd February 2011) to relevant stakeholders and Defra outlining the methods, data, assumptions, results and overall conclusions (Milestone 3).

2. Methods

2. 1 Hazard identification

The first objective of the project (Milestone 1) was a hazard identification in which those viruses that may constitute a hazard in Category 3 ABPs (i.e. present in meat, raw milk, eggs, catering waste and blood) and can infect humans and livestock were identified. This was achieved by combining lists of public and animal health viruses published by the Office of International Epizootics (OIE), the Health Protection Agency (HPA) (Infections A-Z) and Defra (Veterinary Surveillance A-Z). From the initial list, only viruses that infect humans, livestock (cattle, sheep, goats, pigs, and poultry), horses and rabbits were selected. Some of the viruses identified represented large families (e.g. adenovirus, astrovirus) and were split into their main viral hosts (e.g. human, bovine, ovine and porcine). After compiling the list, additional viruses and genuses were added by consulting the taxonomy tree published by the International Committee on Taxonomy of Viruses (www.ictvonline.org) Infectious pancreatic necrosis virus (IPNV) and bovine parvovirus were included as an example of a fish disease and as the reference virus in EC 208/2006, respectively. This yielded a list of 96 viruses.

As the focus of the research was to identify those viruses that could be a relevant hazard in composting and biogas processes, only non-enveloped viruses were considered further. Viruses with a phospholipid envelope are more susceptible to heat and therefore were not considered further. This provided a list of 33 viruses. In discussions with experts in VLA Virology, 20 viruses were selected for further study and development of thermo-stability plots. In reducing the list from 33 to 20 viruses, viruses not likely to be present in Category 3 material were excluded. This included those viruses which only infect humans such as human rotavirus, hepatitis A virus,

human papillomavirus, Norwalk virus, and erythrovirus, as it was considered unlikely that human faeces and tissues would occur in Category 3 material. African horse sickness virus was excluded because, although it may be transmitted via meat to dogs (Anon, 1997), horse-meat is unlikely to be present in Category 3 waste in GB. Epizootic haemorrhagic disease virus was excluded on the basis that the disease is currently not present in Europe and the main route of transmission is through biting midge vectors. Bluetongue virus was selected as a representative virus for those reoviruses transmitted by midges. Swine vesicular disease virus was selected as a representative enterovirus as it is known to be relatively heat resistant (Gale, 2002). Of the adenoviruses, porcine adenovirus was chosen as it is also considered to be relatively heat resistant.

The final list of 20 viruses (adenovirus, astrovirus, avian circoviruses, avian parvovirus blue tongue virus, bovine parvovirus, bovine rotavirus, canine parvovirus, feline calicivirus, foot and mouth disease virus, Hepatitis E virus, infectious bursal disease virus, infectious pancreatic necrosis virus, other calciviruses, other circoviruses, porcine circovirus type 2, porcine parvovirus, rabbit haemorrhagic disease virus, and swine vesicular disease virus) includes at least one virus from each of the following families, namely calicivirus, parvovirus and circovirus. In addition to these viruses, *Salmonella senftenberg* (775W, H2S negative) was also included as the specified reference bacterial indicator organism in Commission Regulation (EC) 208/2006. In undertaking the research for this project, it was noted that there were no data available to produce a thermo-stability plot for "Other circoviruses" and also avian parvoviruses. Therefore, these viruses were removed from the list giving a final total of 18 viruses.

2.2 Thermo-stability plots

From the literature review, decimal reduction times (DRT or D-values, the time for a 1-$\log_{10}$ inactivation) and the times for a 3-$\log_{10}$ reduction were obtained for a range of temperatures and in various media. For *S. senftenberg* (775W, H_2S negative), the time for a 5-$\log_{10}$ reduction was also sought in accordance with the EU Regulations (EC 208/2006). The data within the published studies were highly variable with some studies reporting only 1-log reductions, for example, and others reporting less than or greater than 3-log reductions in various media at the various temperatures. To analyse the data in a consistent and transparent manner, several guidelines were agreed upon by the project team.

To account for the fact that the relationship between the time taken for a 1 to 3 log reduction and temperature may not necessarily be linear for the pathogens studied, two thermo-stability plots are generated: one plot for the time taken for a 3-log reduction and a second plot for the time taken for a 1-log reduction. This approach meant that the time taken for a reduction in viral titre would not be under-estimated but rather, conservatively, may be over-estimated. Further, for viruses which had limited information at the 3-log reduction level, they could still be ranked (or compared) with other viruses' thermo-stability at the 1-log reduction level. Importantly, values that were extrapolated at the 1-log reduction level were visibly separated on the plots from published 1-log values.

It was also noted during the literature review that there were several different media used in the experimental studies and these appeared to have an effect on the thermo-stability of the viruses. To account for this, the media in the literature were broadly categorised into 1) aqueous (e.g. water, buffer or serum), 2) other (e.g. slurries and manures) and 3) meat.

The literature review also highlighted that the data availability varied widely; producing data from different virus strains, different media types and different study protocols. However, in order to compare the relative thermo-stabilities of the selected viruses with that of *S. senftenberg*, it was necessary to combine all of the available data from each of the three media categories. After plotting all these available data points for both the 3-log and 1-log reductions, a line of best fit was plotted using Microsoft Excel 2003 (© Microsoft). This assumes that the studies, virus strains and varying media types within the defined media categories are equally weighted. It is noted that there will be uncertainty in the actual value of the time for the 3-log (or 1-log) inactivation presented for each temperature. For example, the 3-log reduction values taken from inactivation curves presented in the published literature will have uncertainty based on the methods by which the curves were fitted to the experimental data. Uncertainty will also be present in the extrapolated 3-log values that were obtained from larger log reductions given in the literature. No uncertainty analysis has been undertaken here for the inactivation times.

The availability of the data also has a direct impact on the fit of the best-fit line to the data and the assumed relationship for that fit (e.g. linear, exponential). A virus with few data points (e.g. n=2) will produce a line with a very good fit to the data (i.e. R^2=1) whereas a virus with many highly variable data points (n) may produce a less well fitting line. The R^2 value for each virus and *S. senftenberg* is summarised for each thermo-stability plot. For all plots, a linear relationship between the points is assumed over the temperature range. The objective of the work presented here is not to investigate the slope of the line (i.e. the relationship between inactivation time with temperature). The line of best fit, as used here, attempts to represent the mid-points of the inactivation for each temperature and, in this respect, fulfils the objective of the work by facilitating comparison of the relative positions of the line for each pathogen.

As the data for each of the three media were plotted separately, there are up to 6 best fit lines for the 1-log reduction thermo-stability plot (3 lines representing *published* D-values per media and 3 lines representing *extrapolated* D-values per media) and 3 best fit lines for the 3-log thermo-stability plots. It is important to note that those data points that were estimated from a zero-log reduction are not included in the line of best fit and are denoted by an "x" on the plots due to the high level of uncertainty associated with these D-values. These data points remain in the plots to ensure all the available data is included.

3. Results

Thermo-stability plots were generated for each of the viruses under study and *S. senftenberg* (Milestone 2 report). In each of these plots, a line of best fit was fitted to the data and used to provide an indication of the relative thermo-stability between the viruses and the reference bacterium, *S. Senftenberg* (775W, H_2S). Specifically, the lines of best fit for a 3-log reduction in "aqueous" media for each virus were plotted (see Figure 1). "Aqueous" media was selected as data were available for that medium group for all pathogens studied except for hepatitis E virus whereby "other" media is used so that all pathogens can be compared. Rabbit haemorrhagic disease virus is not included as no data were available for a 3-log reduction. Indeed for this virus, the only study available reported it was still infectious to rabbits after 2 days at 60°C. In generating the plot in Figure 1, the lines of best fit were not extrapolated to include the entire range of temperatures under study; each line only covers the range of temperatures for which data were available. It was considered that extrapolating to the full range of temperatures may lead to incorrect conclusions.

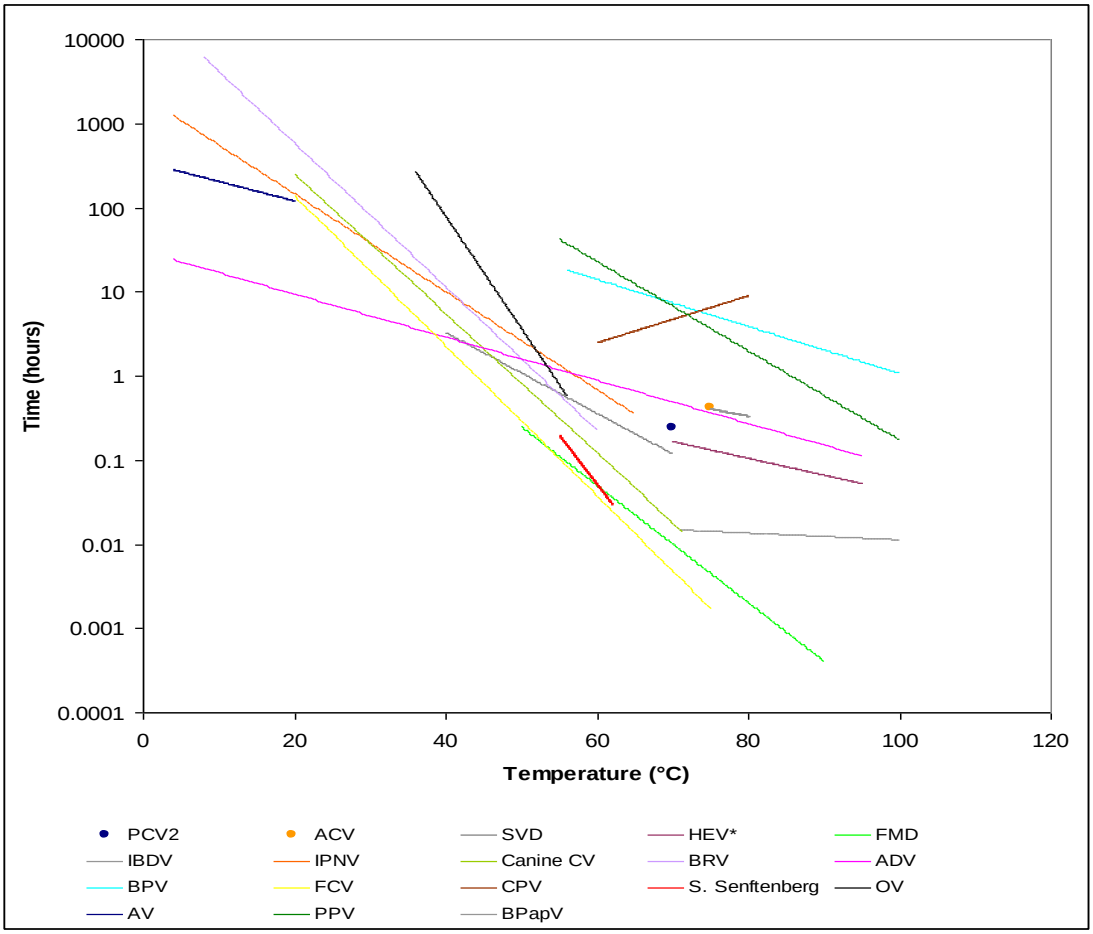


Figure 1: Thermo-stability plot: Times for a 3-log reduction of the viruses and for a 5-log reduction of *Salmonella senftenberg* (775W, H_2S) as a function of temperature in aqueous media

The best fit line for canine parvovirus (CPV) increases with temperature in contrary to other viruses but this is mainly due to the fact that the best fit line is based on only 3 data points relating to 3 different virus strains from 3 separate scientific studies. It is apparent in Figure 1, that bovine parvovirus (BPV) and porcine parvovirus (PPV) are the most thermo-resistant pathogens; i.e. take the longest time to achieve a 3-log reduction for the temperature of interest.

A similar analysis can be undertaken at the 3-log reduction level for those pathogens for which data are available on "other" media. The resulting plot is outlined in Figure 2.

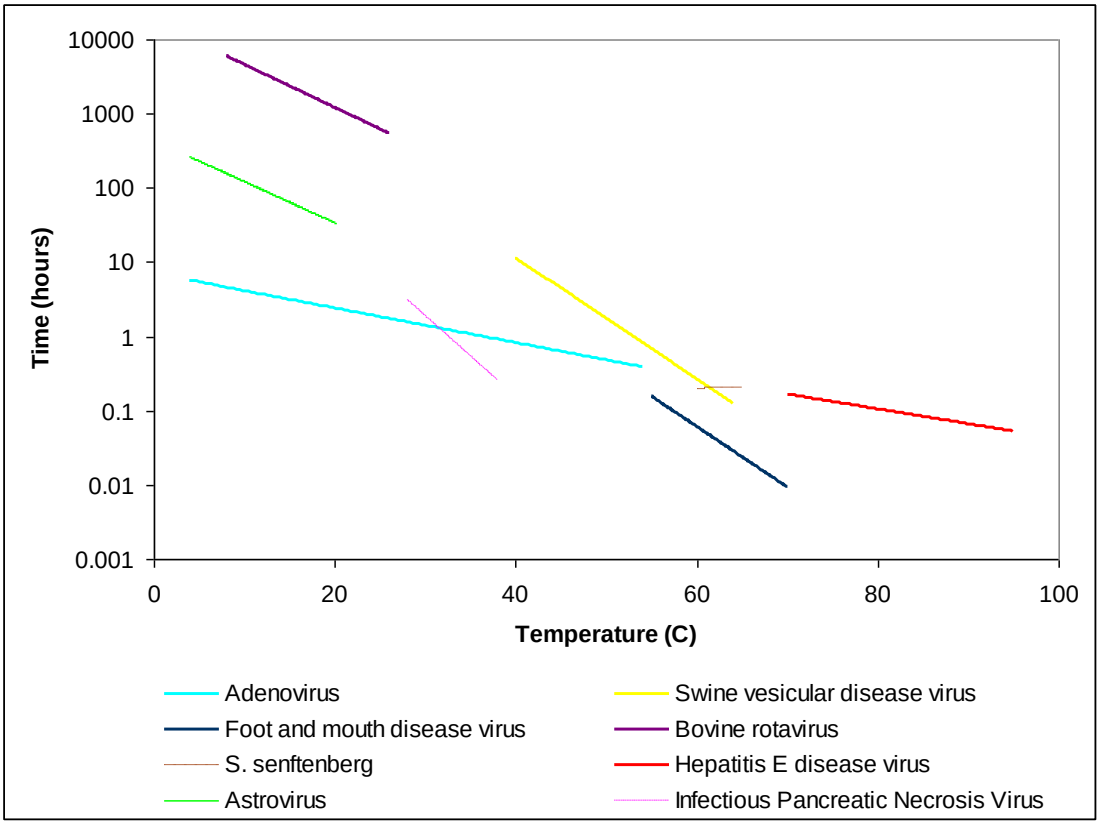
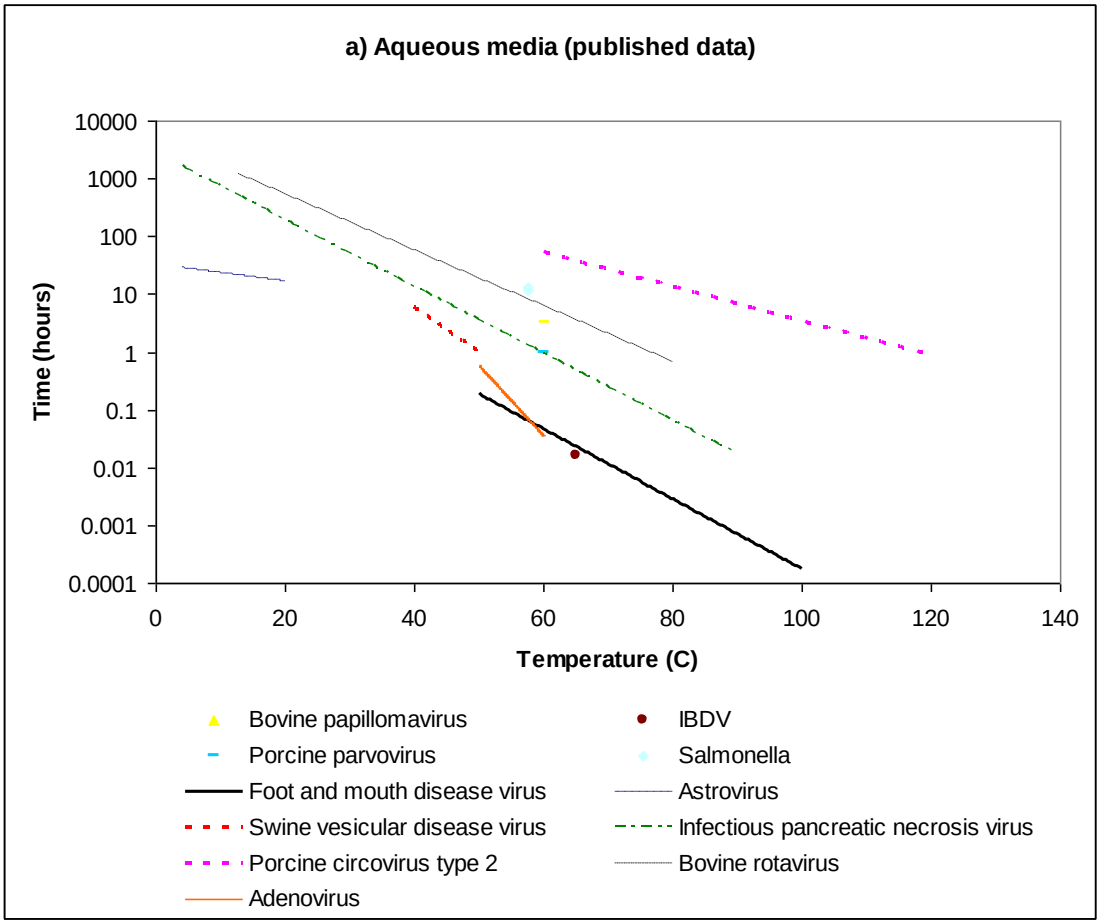


Figure 2: Thermo-stability plot: Times for a 3-log reduction of the viruses and for a 5 log reduction of *S. senftenberg* (775W, H_2S) as a function of temperature in "other" media

It can be seen in Figure 2 that there are data available for "other" media for 7 viruses; no data are available for bovine parvovirus (the reference virus) in "other" media. There is limited overlap between the pathogens in the temperature ranges for data are available; however, of these 7 pathogens, Hepatitis E virus appears visually to be one of the more thermo-resistant pathogens.

For "meat" media, there are only three pathogens for which data are available at the 3-log reduction level (*S. senftenberg*, foot and mouth disease virus (FMDV) and infectious bursal disease virus (IBDV)). Of these, infectious bursal disease virus and foot and mouth disease virus are more thermo-resistant than *S. senftenberg*.

In addition, a comparison of the viruses and *S. senftenberg* at the 1-log reduction level in "aqueous" media for both published and extrapolated data was undertaken and is illustrated in Figure 3.



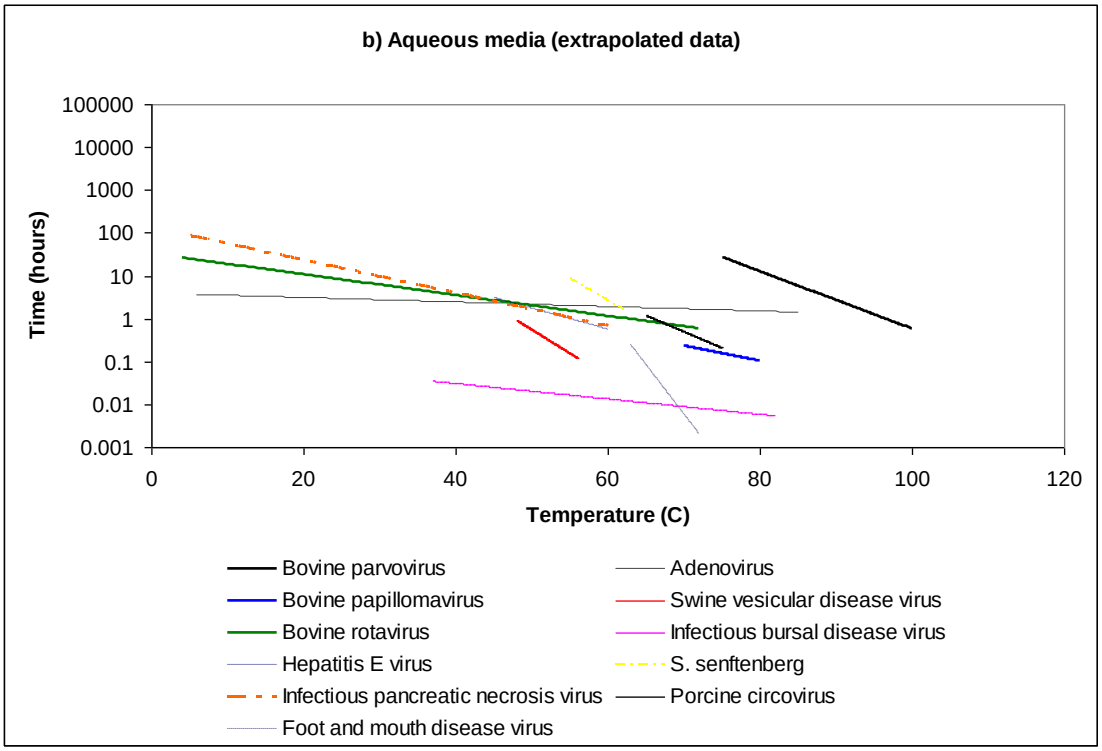
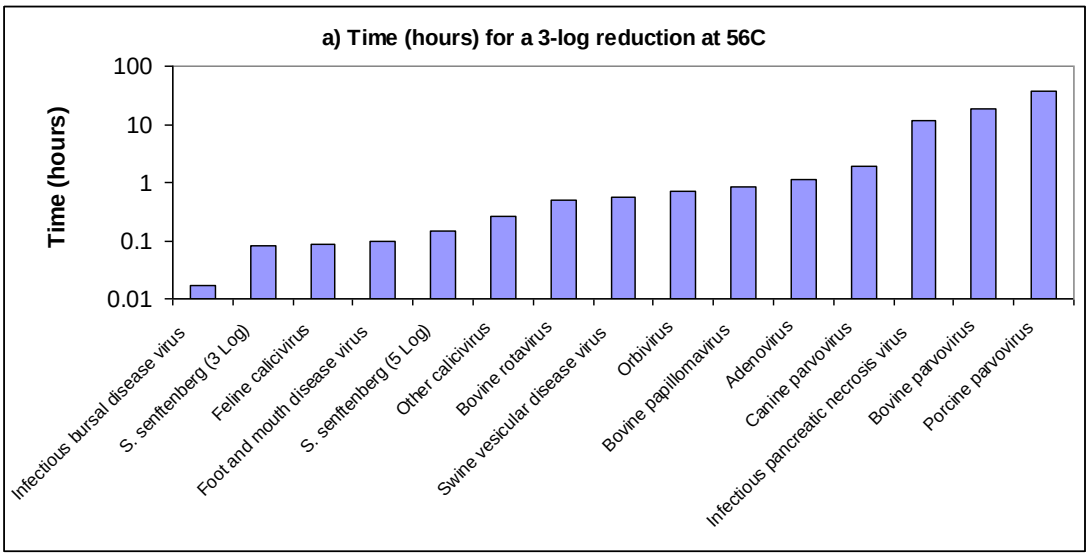
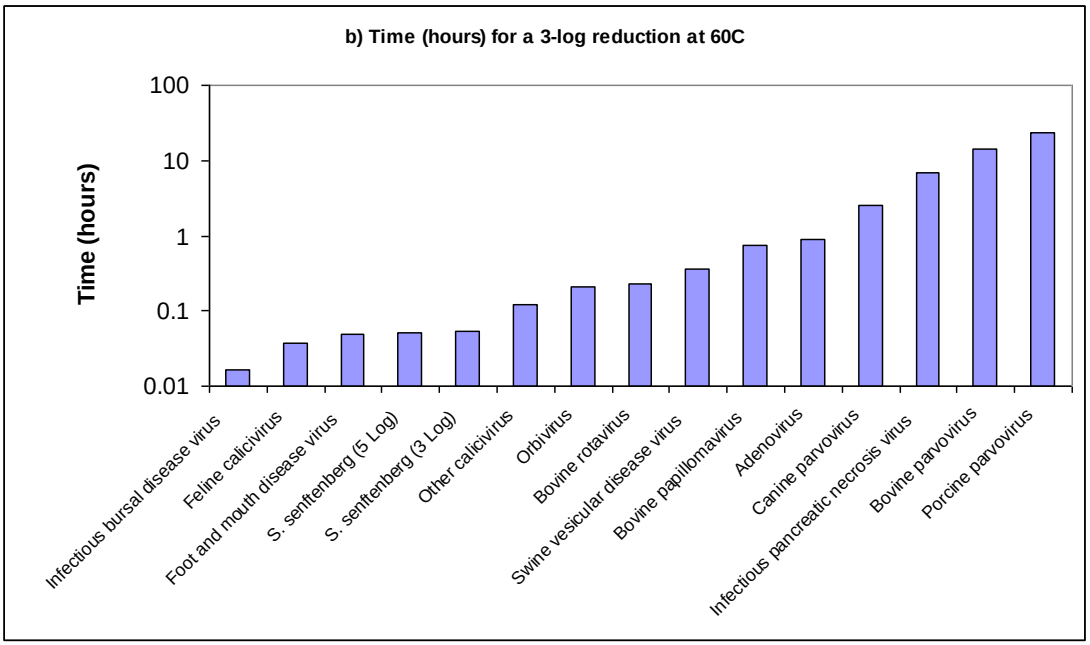


Figure 3: Thermo-stability plots: Times for a 1-log reduction of the viruses and *Salmonella senftenberg* (775W, H_2S) as a function of temperature in aqueous media

It can be seen in Figure 3 b) that bovine parvovirus takes the longest time to achieve a 1-log reduction in aqueous media based on data estimated using extrapolation techniques; this is in agreement with the 3-log reduction conclusions. When considering only published data (Figure 3 a)), it is porcine circovirus type 2 that exhibits the most thermo-resistant profile. However, there are no published data available for the parvoviruses with which to compare.

Using the fitted trend line, the number of hours it would take to obtain a 3-log reduction at 70°C, can be estimated for all pathogens except Rabbit haemorrhagic disease virus. This would enable a more quantitative comparison and ranking between the pathogens. In doing so, however, it is assumed that the data appropriately fits a linear trend for each virus and *S. senftenberg* and can be extrapolated to this temperature. Based on the R^2 value for some pathogens for "aqueous" media, however, it is observed that the data does not always fit a linear trend particularly well. Given this important caveat, the rankings of the viruses at 56°C, 60°C and 70°C in "aqueous" media with respect to the times taken for a 3-log reduction using the available data (i.e. published and extrapolated) are summarised in Figure 4.





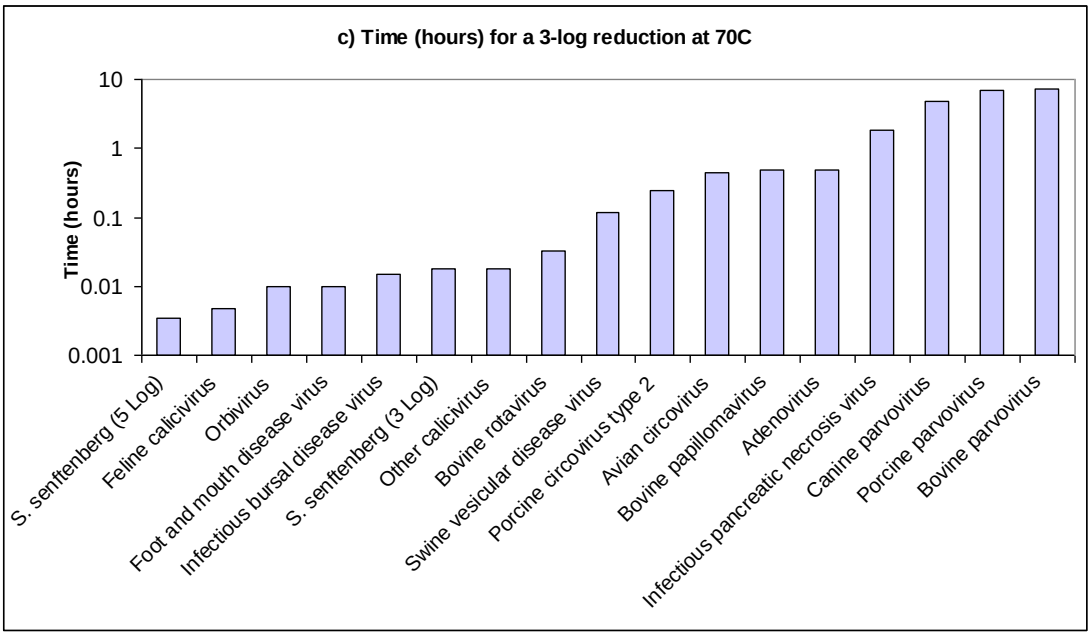


Figure 4: Time (hours) for a 3-log reduction for each virus and *S. senftenberg* in aqueous media; a) at 56°C, b) at 60°C, and c) at 70°C

In interpreting Figure 4, several points should be noted:

- for Hepatitis E there are no data available for “aqueous” media so the pathogen has not been included in the analysis.
- for avian circovirus the only data point available was for 75°C rather than 70°C. Therefore, this value was used in Figure 4 c) and not included in Figure 4 a) or b).
- astrovirus has not been included in Figure 4 as data were only available for temperatures below 25°C. It was considered that extrapolating the trend line to temperatures greater than or equal to 56°C would provide inaccurate conclusions.
- The following pathogens do not include available data at either 56°C, 60°C or 70°C so the trend line has been extrapolated to estimate the number of hours required for a 3-log reduction at these temperatures:
    - 56°C - infectious bursal disease virus, *S. seftenberg* (at the 5 -log reduction level), bovine papilloma virus, canine parvovirus and bovine parvovirus.
    - 60°C - infectious bursal disease virus, *S. seftenberg* (at the 3 -log reduction level), orbivirus and bovine papilloma virus.
    - 70°C - *S. senftenberg*, orbivirus, bovine rotavirus, bovine papillomavirus, and infectious pancreatic necrosis virus.

Given the caveats with the methodology, it can be seen from Figure 4 that the parvoviruses (porcine and bovine) are the most thermo-resistant pathogens at 56°C, 60°C and 70°C. The EC Regulations specify that the chemical and thermal processes can be validated by testing for at least a 3-log reduction in infectivity titre of thermo-resistant viruses such as parvovirus. The Regulations further specify that particles may be treated at 70°C for at least an hour. The research conducted here, however, suggests that using the fitted trend line, parvovirus requires at least 5 hours at 70°C in order to observe a 3-log reduction. Based on the data within Figure 4, it is estimated that in one hour at 70°C, all pathogens except for infectious pancreatic necrosis and parvoviruses will have at least a 3-log reduction in infectivity.

The impact of media (i.e. “other” versus “aqueous”) on the thermo-stability of the pathogens can also be assessed using the above approach for those pathogens whereby data are available on “other” media. This analysis is shown in Figure 5.

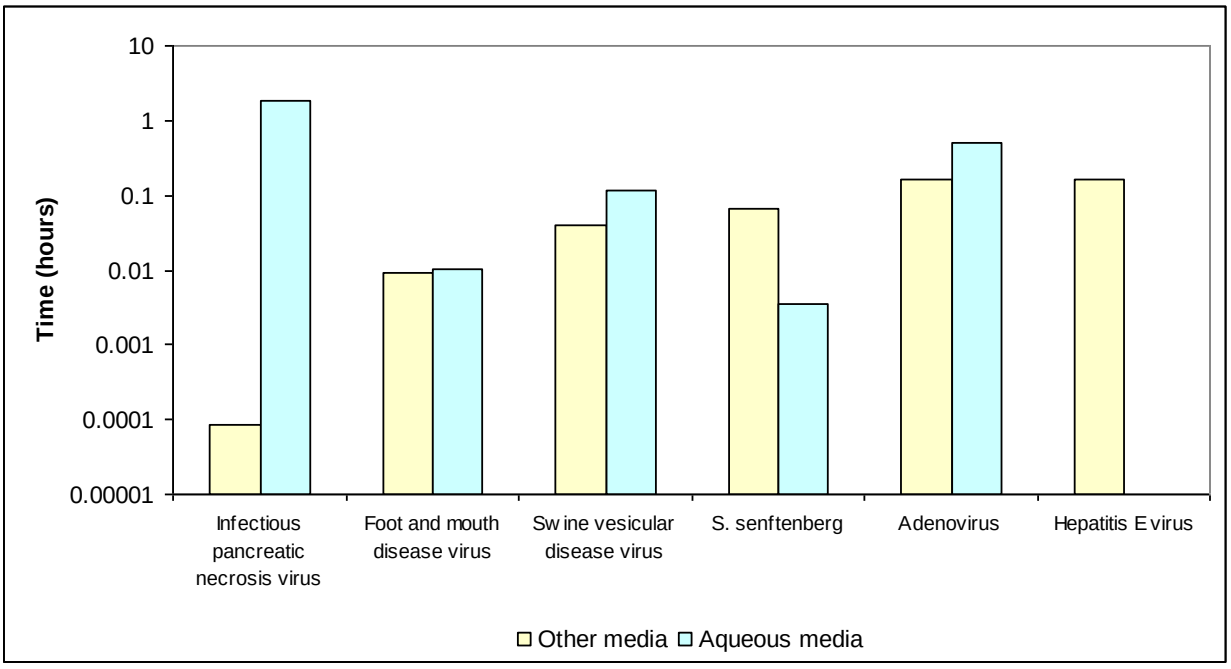


Figure 5: Comparison of the time (hours) for a 3-log reduction at 70°C in “aqueous” and “other” media for pathogens where data are available

As observed in Figure 5, infectious pancreatic necrosis virus is the most thermo-resistant in “aqueous” media. However, it is the least thermo-resistant in “other” media. In the latter media, Hepatitis E virus is the most thermo-resistant; however as data are not available for Hepatitis E virus in “aqueous” media, it can not be ascertained how thermo-resistant the pathogen is in “aqueous” media. Further data are required to ascertain the impact of media on all of the pathogens, particularly the reference pathogen (bovine rotavirus).

For meat media, there are only three pathogens (FMDV, IBDV, and *S. senftenberg*) for data were available. However, it was observed that FMDV and IBDV are more thermo-resistant in “meat” compared to “aqueous” media suggesting that meat may have a protective effect.

4. Main implications of study and conclusions

The first aim of the project was to identify a list of viruses which may be present in Category 3 ABPs and be thermo-resistant. A list was compiled of 18 viruses for which data were gathered on their thermo-stability in different media and at varying temperatures. The second aim of this project was to generate thermo-stability plots of the selected viruses and *S. senftenberg* thus enabling a ranking of the relative thermo-stabilities. Based on the caveats of the available data (i.e. variation in strains, media types, data availability), it is challenging to definitively rank the viruses' thermo-stability. However, based on the data available (hence the data constraints) comparing the thermo-stability of 18 viruses and *S. senftenberg* (Figures 1 & 4), some overall conclusions can be drawn:

- media type has an impact on the thermo-stability of the selected viruses with, in general, thermo-stability of the viruses and *S. senftenberg* being highest for meat then aqueous medium and finally other media.
- parvoviruses (canine (CPV), bovine (BPV) and porcine (PCV)) appear to be the most thermally resistant and are clustered closely on the thermo-stability plots.
- it is estimated that in one hour at 70°C, all pathogens except for infectious pancreatic necrosis and parvoviruses will have at least a 3-log reduction in infectivity in aqueous media.
- Given the data caveats and assumptions, *S. senftenberg* is not an appropriate marker for ensuring that a 3-log reduction has been achieved in viral hazards potentially present in Category 3 ABPs.
- Parvovirus, particularly bovine and porcine, is an appropriate marker for ensuring that a 3-log reduction has been achieved in viral hazards potentially present in Category 3 ABPs.

A primary conclusion, given the caveats on data quality and availability, is that this research agrees with the Commission Regulation (EC) 208/2006 and Regulation EC 1774/2002 in that testing for a 3-log reduction in parvovirus (in aqueous media) is likely to ensure that other viruses (particularly those identified in the hazard identification process) will have a greater or similar log reduction at the validation temperature.

5. Possible future work

In conducting this research, it is apparent that there is high degree of variation in the availability of data on the thermo-stability of the 18 selected viruses. This has impacted on the ability to produce the plots and compare the different pathogens without introducing assumptions regarding the available data. In order to verify the conclusions from this research, it would be beneficial to undertake further research aimed at limiting the data gaps, particularly for certain pathogens such as canine calicivirus, for example. Further analysis would also be required to ascertain the impact of media on the thermo-stability of the pathogens under study on the conclusions drawn particularly for the parvoviruses – i.e. can the same conclusions be drawn for meat media, for example.

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

The relative thermo-stability of selected viruses that pose a hazard in Category 3 Animal By-Products used as incoming materials in biogas and composting plants. Rowena Kosmider, Neil Donaldson, Dave Selby, Nick Reed, Paul Gale & Emma Snary. Journal for Applied Microbiology. Manuscript in preparation.