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Pig carcass decomposition dynamics: Insights into carcass disposal for emergency animal disease management

O Schlosberg,[a] BD Cowled,[a] J Torpy,[a] A Höger,[a] RE Thompson,[b] TS Barnes,[c,d] K Richards,[e] RN Hall[a] and EEC Leslie[a]*

Australia is free from many economically devastating emergency animal diseases (EADs) that threaten livestock production in neighbouring countries. In Australia, an important consideration for EAD control is managing susceptible feral animal populations, especially in remote and inaccessible areas where carcass disposal poses considerable logistical challenges. One proposed solution is to utilise natural decomposition above ground through the 'destroy and let lie' (D&LL) method, relying on post-mortem changes in carcass temperature and pH to inactivate the EAD agent. We investigated temperature and pH changes in pig carcasses from death until end-stage decomposition at two locations in Queensland to gain insights into how carcasses left *in situ* decompose under Australian conditions. Using regression modelling, we identified days since humane killing, air temperature, rainfall, relative humidity, anatomical site and study location as significant predictors of carcass pH and temperature. Although the observed carcass pH and temperature conditions did not meet African swine fever virus (ASFV) inactivation thresholds, foot-and-mouth disease virus (FMDV) was likely to be inactivated in the thoracic cavity, superficial and deep skeletal muscle and abdominal cavity of most carcasses. However, FMDV inactivation thresholds were not reached in bone marrow and brain. This suggests that these carcasses may potentially remain infectious with ASFV and FMDV *in situ* under the experimental conditions encountered, based on the inactivation thresholds selected. Despite this, culling large portions of a feral pig population, in conjunction with D&LL disposal approach, may still support disease control imperatives during an EAD response by reducing live pig numbers and disease transmission.

Keywords carcass; decomposition; disposal; emergency animal disease; pig

Abbreviations ANOVA, analysis of variance; ASF, African swine fever; ASFV, African swine fever virus; AUSVETPLAN, Australian Veterinary Emergency Plan; D&LL, destroy and let lie; EAD, emergency animal disease; FMD, foot-and-mouth disease; FMDV, foot-and-mouth disease virus; GAM, generalised additive model; HSD, honestly significant difference; IQR, interquartile range; Q1, quartile 1; Q3, quartile 3; RF, random forest; WOAH, World Organisation for Animal Health

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*Corresponding author.
[a]Ausvet Pty Ltd, Fremantle, Australia; edwina.leslie@ausvet.com.au
[b]Biosecurity Queensland, Queensland Department of Agriculture and Fisheries, Nambour, Australia
[c]Epivet Pty Ltd, Withcott, Australia
[d]School of Veterinary Science, The University of Queensland, Gatton, Australia
[e]SunPork Group, Eagle Farm, Australia

African swine fever (ASF) and foot-and-mouth disease (FMD) are two economically devastating transboundary animal diseases. The global distribution of these diseases has changed recently, with Indonesia experiencing incursions of ASF in September 2019 and FMD in April 2022, and ASF emerging in Timor-Leste in September 2019 and in Papua New Guinea in March 2020. As a result, Australia has increased its preparedness activities and reviewed its response plans to ensure that strategies in the event of an outbreak are appropriate for the context in which they will be applied.

Emergency animal disease (EAD) responses in Australia are guided by the Australian Veterinary Emergency Plan (AUSVETPLAN), a nationally agreed approach to EAD control. If an outbreak of either ASF or FMD were to occur in Australia, feral pigs (*Sus scrofa*) may be epidemiologically involved and complicate control and surveillance efforts, as they are susceptible to both diseases and have large, widely dispersed populations in Australia, estimated at approximately 3.2 million in the 2000s.[1] The role of wild boar in ASF epidemiology is well documented in the literature in the European context, and they have also been considered a threat for FMD transmission.[2,3] Epidemiological models examining the potential role of feral pigs in an EAD outbreak in Australia have suggested that, although infection may eventually fade out in feral pig populations, control would still be warranted as it would lead to faster disease elimination and would reduce spillover events between feral pigs and domestic livestock that may contribute to ongoing disease transmission.[4–6] Feral animals pose a major logistical challenge for EAD responses in Australia, especially those populations located in rugged and remote areas.

As well as disease-specific documents, AUSVETPLAN also includes operational manuals that detail recommended procedures for activities that are common to most EAD responses. One such document is the Disposal manual, which provides a decision-making framework for waste disposal for disease control purposes, including both animal carcasses and products.[7] Disposal methods detailed in this manual include burial, burning, rendering, composting and anaerobic digestion.[7] However, the application of these methods is limited in extensive areas of Australia that have populations of remote livestock or feral animals and are logistically challenging to access and muster.[7] A potential alternative carcass disposal method that has been proposed for these areas is where culled animals remain *in situ* and carcasses are left to decompose above ground under natural conditions, the so-called 'destroy and let lie' (D&LL) method.[7] This approach relies on post-mortem changes, including carcass temperature and pH conditions, to reduce infectivity of the EAD agent where it may be present.

Although preliminary studies have suggested that D&LL may be a viable approach in specific contexts,[8–10] further research is needed to assess its generalisability across different climatic regions and to validate the method.[7] This is especially true because ASF maintenance has been associated with wild boar carcasses in temperate regions.[2]

Carcass decomposition has been extensively studied under refrigerated conditions for meat science and food safety purposes. However, studies evaluating pathogen inactivation have typically utilised disposal methods such as composting and burial.[11–19] Limited information is available on pig carcass decomposition *in situ*, particularly under Australian conditions.[8–10] Previous Australian studies investigating the utility of the D&LL disposal method were restricted to examining pH and temperature changes in animal carcasses for 48 hours post humane killing. pH changes consistent with the possibility of foot-and-mouth disease virus (FMDV) inactivation were observed in the body cavity and in deep and superficial muscle of most carcasses, but not in brain or bone marrow.[8–10]

We expanded on this prior research by extending the period of carcass monitoring until end-stage decomposition. We investigated temperature and pH changes over time in pig carcasses at two locations in Queensland (eastern Australia) to gain insights into how carcasses left *in situ* decompose under Australian conditions. Specifically, the study objectives were to (1) understand how pig carcass temperature and pH change over time under different environmental conditions, 2) determine the factors that influence decomposition rates and 3) evaluate if internal physiological parameters become conducive to pathogen inactivation over extended timeframes. This information is important to understand how D&LL may be applied in Australian conditions.

Materials and methods

Study locations

Decomposition trials were conducted at two commercial pig farms in Queensland, Australia, one in Warra in the Western Downs region of inland southeast Queensland and one in Biloela in the Shire of Banana in central Queensland (Supporting Information). Two trials were conducted at each location, totalling four trials. Fieldwork took place in the southern hemisphere summer and early autumn (wet season), between November 28, 2023, and April 2, 2024. Further information about the study sites is given in the Supporting Information file.

Animals

Each trial used eight pig (*Sus scrofa domesticus*) carcasses. Grower pigs (Landrace, Large white cross) aged 10–16 weeks of mixed sexes and weights ranging between 30 and 80 kg were included. Sample size calculations are described in the Supporting Information file.

Pigs were humanely killed using a single captive bolt as required by animal ethics (Warra: BK-014 Blitz Kerner Captive Bolt Pistol, Everything ID; Biloela: CASH Special Captive Bolt Stunner.22 Calibre Heavy Duty 4000, FPE – Food Processing Equipment Ltd), with death confirmed by vital signs assessment. We use the term 'humane killing' instead of euthanasia, in line with the recommendations of the 'Australian code for the care and use of animals for scientific purposes' and the Australian Veterinary Association. Each carcass then received a gunshot to the head with a 0.22 calibre rifle, to simulate aerial shooting as a depopulation method during an EAD response. Although this is a smaller calibre than would be used in Australia during an EAD response, the calibre selection was chosen to align with the previous D&LL study[9] and to minimise carcass damage. Additionally, three carcasses per trial (total of 12 animals) received an abdominal shot with a 0.308 calibre rifle, to investigate the potential effects of internal organ disruption on decomposition parameters. The 0.308 calibre aligned with the 'Standard Operating Procedure for Aerial Shooting of Feral Pigs'.[20] Each carcass was positioned in lateral recumbency, approximately 1 metre apart, within a fenced enclosure to prevent access by scavengers (Supporting Information).

Ethical statement

All work was conducted in accordance with the 'Australian code for the care and use of animals for scientific purposes'. Animal ethics approval for this research project was provided by Queensland Government Department of Agriculture and Fisheries Animal Ethics Committee (#CA 2023/10/1796).

Carcass monitoring and data collection

Automated carcass temperature and pH recordings were collected hourly using commercially available sensors (WP-80 or WP-80 M units, TPS, Queensland, Australia) inserted into six anatomical sites: abdominal cavity, bone marrow, brain, deep muscle, superficial muscle and thoracic cavity (Supporting Information). A two-point pH calibration was undertaken every 5 days, calibrated against pH 4.0 and pH 7.0 buffer solutions. Temperature and pH sensors were cleaned and charged every 2 days. Instruments were housed in modified cooler boxes to limit exposure to ambient conditions. Pig carcasses were monitored visually daily for the first 2 days and then inspected every 1–2 days for the duration of each trial. Carcass inspection included documenting decomposition progression and insect activity and correcting sensor placement as needed (especially during early stages where gas build-up caused sensor displacement).

Stages of decomposition were defined according to a modified model that identifies six distinct stages for carcass decomposition in pigs.[21] For this study, we combined the 'dry' and 'remains' stages into a single category, resulting in a five-stage classification: fresh/bloated, bloated, active decay, advanced decay and dry/remains (Table 1).

Trials were concluded when there was complete soft tissue decomposition and bone exposure, or extensive soft tissue decomposition accompanied by mummified tissue, and no observed changes in decomposition for a minimum of three consecutive days. The pH data were also downloaded from the sensors and analysed alongside monitoring carcass changes to ensure that values had stabilised and were not substantially changing.

Environmental data collection

Weather data relevant to each study location were obtained from the Australian Bureau of Meteorology weather stations located at Dalby Airport (station number 041522) for the Warra location (approximately 45 km away) and Thangool Airport (station number 039089) for the Biloela location (approximately 23 km away). No closer weather stations were available with an hourly recording frequency.

Table 1. Decomposition stage descriptions for pig carcasses.

Stage	Observation criteria
Fresh/bloated	Minimal discolouration, rigor mortis present, carcass temperature approaching air temperature.
Bloated	Distension of body cavities, seepage of fluids from natural body openings, strong smell of ammonia.
Active decay	Breaking of the outer layer of the skin, escape of gasses from the abdomen, carcass collapse, strong odours of decomposition.
Advanced decay	Soft tissue largely decomposed, odours of decay beginning to fade.
Dry/remains	Dried skin and cartilage, hair and bone remain.

Adapted from the model established by Payne (1965).[21]

Data cleaning and outlier removal

Data were inspected visually using ggplot2_3.5.1 as implemented in R versions 4.2.0 and 4.3.1. Residual analysis and a modified Tukey's method were used to identify outlying replicates from temperature and pH time series data, split by trial and anatomical site. Additional details are given in the Supporting Information file.

Measurements from at least three pigs were required at each timepoint. For timepoints with fewer than three values, all datapoints from the two timepoints immediately preceding and two timepoints after the timepoint were used in addition to the datapoints of the timepoint to calculate the median. Datapoints with a pH difference of 1 or more from the median were considered outliers and removed.

All analyses were conducted in R versions 4.2.0 and 4.3.1 using the packages cowplot_1.1.3, dplyr_1.1.4, ggplot2_3.5.1, ggpubr_0.6.0, ggspatial_1.1.9, gridExtra_2.3, gtools_3.9.5, hms_1.1.3, leaflet_2.2.0, lubridate_1.9.3, maps_3.4.1, naturalsort_0.1.3, purrr_1.0.2, RColorBrewer_1.1–3, readxl_1.4.3, sf_1.0.14, stringr_1.5.1 and tidyr_1.3.1.

Descriptive statistical analyses

Statistical significance was evaluated using analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test or the Mann–Whitney U test. Statistical significance was evaluated at $P < 0.05$.

Statistical modelling

Relationship of carcass temperature and pH with predictors. Because descriptive analyses suggested nonlinear relationships between pH and the various predictors under investigation, a generalised additive model (GAM) was fitted. The GAM had a Gaussian distribution and identity link function. Smooth terms with cubic regression splines were used for the continuous predictors (days since humane killing, air temperature, rainfall and humidity). Animal identifier was included as a random effect and used a random effects spline. Categorical predictors (sex, anatomical site and study location) were included as parametric terms. The final pH GAM was

$$\begin{aligned}\text{Carcass } pH = {} & B_0 + S_1\text{Days since humane killing} + S_2\text{Air temperature} \\ & + S_3\text{Rainfall} + S_4\text{Animal identifier} + S_5\text{Humidity} \\ & + B_6 Sex + B_7\text{Anatomical site} + B_8\text{Study location} + \mathcal{E}\end{aligned}$$

When analysing the impact of including an abdominal shot on carcass decomposition, we added an extra fixed effect representing whether each pig carcass received an abdominal shot (and a head shot) or just a head shot.

We examined residuals to evaluate the model. Influential outliers were identified using Cook's distance. Model goodness-of-fit was evaluated using adjusted R-squared and the percentage of deviance explained by the model. The generalised cross-validation criterion was used to assess model complexity and potential overfitting. Smooth-term visualisations were generated to visualise the estimated relationships between the continuous predictors and pH.

After the implementation of the GAM, biologically plausible outliers remained that violated some model assumptions. We also wished to make more accurate predictions than were possible with the regression model. We therefore used machine learning to implement random forest (RF) algorithms. RF models are nonparametric; are more robust to outliers, unmodelled interactions, collinearity, clustering and overfitting; and are likely to be more accurate for predictions than regression models.[22,23] Details of model fitting, training and tuning are given in the Supporting Information file.

To investigate the relationship between temperature and various predictors, we fitted a linear mixed effects model with animal identifier as a random effect term. The error term was assumed to follow a Gaussian distribution. Humidity and air temperature were strongly collinear, so humidity was removed from the final model. The final temperature linear model was

$$\begin{aligned}\text{Carcass temperature} = {} & B_0 + B_1\text{Days since humane killing} \\ & + B_2\text{Air temperature} + B_3\text{Rainfall} + B_4 Sex \\ & + B_5\text{Anatomical site} + B_6\text{Study location} \\ & + (1|\text{Animal identifier}) + \mathcal{E}\end{aligned}$$

As described above for the pH model, an extra fixed effect was added when analysing the impact of including an abdominal shot.

Model assumptions and performance were evaluated using ANOVA and standard diagnostic plots. Machine learning was implemented to enable more accurate predictions, as described above for the pH RF model.

All analyses were conducted in R version 4.2.0[24] using the packages caret_6.0–94,[25] lme4_1.1–35.3,[26] mgcv_1.8–40[27] and randomForest_4.7–1.1.[28]

Viral inactivation thresholds

Viral inactivation thresholds for African swine fever virus (ASFV) and FMDV were guided by the World Organisation for Animal Health (WOAH), AUSVETPLAN, primary inactivation studies from

the literature and previous D&LL investigations (Table 2). A conservative approach in assigning thresholds was used to increase the confidence in viral inactivation inferences. This is described further in the Supporting Information file.

Results

Carcass decomposition

A total of 32 domestic grower pigs (*S. scrofa domesticus*) with an overall mean weight of 52.8 kg (SD = 13.4 kg) were used in the study. Trial durations ranged between 12.1 and 18.2 days (Table 3). Mean air temperatures were similar across the four trials (24.1°C–26.9°C) (Table 4). Mean relative humidity was lower at the Warra location (60.3–71.9%) compared with the Biloela location (70.3–75.2%) (Table 4). Total rainfall varied considerably across the four trials (16.3–818.7 mm) (Table 4).

Decomposition followed a similar pattern across all trials, although the duration of each stage varied (Figures 1 and 2). The fresh/bloated stage lasted 1 day for all trials. The bloated stage lasted between one day (trial 2 and 3) and 2 days (trial 1 and 4). Active decay ranged from two days (trial 2) to 8 days (trial 3). Advanced decay lasted between five days (trial 2) and 12 days (trial 4). The dry/remains stage lasted from two days (trial 3) to 7 days (trial 4). The duration of the final stage reflected the timing of sensor removal, not the natural progression of decomposition. A series of post-mortem morphological changes, including livor mortis, rigor mortis, algor mortis and greenish discoloration, were observed in the decomposition process across all carcasses (Figure 2).

Weather conditions appeared to influence decomposition rates across trials. In trial 1, initial high temperatures, rain and humidity led to rapid decomposition, which slowed as moisture levels decreased. The death of maggots on day 7, associated with high air temperature, further slowed decomposition, promoting mummification of tissues. Trial 2 experienced consistently high moisture levels, which, combined with slightly milder temperatures compared with the previous trial, sustained maggot activity for a longer period, likely contributing to the more rapid progression of decomposition. Trial 3 exhibited fluctuating temperatures and humidity, alongside minimal rainfall. This resulted in early loss of maggot activity by day 7 and a subsequent slowing in decomposition. This led to more tissue mummification. Trial 4 displayed an initial period of high heat and low moisture, reducing maggot activity by day 7 and slowing decomposition. Despite being followed by milder temperatures, higher humidity and continuous rainfall, maggot activity did not resume.

Table 2. Inactivation thresholds used in this study for foot-and-mouth disease virus and African swine fever virus.

Virus	Threshold	Cumulative time (hours)	References
ASFV	pH <3.9	>0	41,42
ASFV	pH >11.5	>0	41,42
FMDV	pH <6	>0	44
FMDV	pH >9	>0	44
ASFV	Temperature ≥56°C	≥2	41
ASFV	Temperature ≥60°C	≥1	41
ASFV	Temperature ≥69°C	≥0	43
FMDV	Temperature ≥43°C	≥7	8
FMDV	Temperature ≥49°C	≥1	8

Carcass and ambient conditions were conducive for insect development across all trials. Subjectively, more insects were observed at the Warra location compared with the Biloela location, although this was not quantitatively evaluated. Subjectively, flies were the sole insect observed during the fresh/bloated stage. Flies were present within the first hour after humane killing in trials 2–4, whereas heavy rain resulted in the absence of flies on the first day in trial 1. Fly colonisation (i.e. the presence of maggots) occurred uniformly across all carcasses in each trial during early decomposition. A second generation of flies emerged during the active decay stage in all carcasses, increasing the number of flies observed. In the absence of professional entomological identification, flies were identified as family Calliphoridae or family Sarcophagidae, both of which are commonly associated with decomposition processes.[24]

Maggot colonisation of the carcasses was observed during the bloated stage, initially concentrated in the head region (eyes, mouth and cranial gunshot wound). Maggot activity peaked during the active decay stage. Maggot activity was largely absent from all carcasses by day 7 (trials 1, 3, 4) to day 11 (trial 2).

Beetles (family Cleridae, genus *Necrobia* or close relative) were present on the carcasses during the bloating stage for trials 1 and 4, and during active decay for trials 2 and 3. No ants were observed on any of the carcasses across all trials. Cane toads (*Rhinella marina*) were observed feeding on maggot populations during trials 3 and 4 on two separate nights. This was primarily during night hours and during periods with peak maggot activity.

Temperature and pH changes by anatomical site

Carcass temperature and pH data were collected hourly at six anatomical sites (i.e. abdominal cavity, bone marrow, brain, deep muscle, superficial muscle, thoracic cavity). A total of 13% of temperature readings and 32% of pH readings were classified as outliers during the data cleaning process (Table 3). The cleaned dataset for all trials comprised 49,039 individual temperature readings and 38,083 pH readings.

Across all trials and time points, carcass pH ranged from 4.7 in the thorax to 9.1 in the bone marrow (Figure 3). Mean pH was lowest in the abdominal cavity, at 6.8. Mean pH at other anatomical sites ranged from 7.1 to 7.4. The lowest values were recorded in the first 48 hours post humane killing (Figure 4). Differences in pH between most combinations of anatomical sites were statistically significant (ANOVA $P < 0.001$) (Figure 3).

Across all trials and timepoints, carcass temperature ranged from 15.5°C to 58.6°C (Figures 5 and 6). The hottest temperatures for trials 1, 2 and 3 were recorded in the last 5 days of each trial, whereas the hottest temperature for trial 4 was recorded on day 7 and appeared to be associated with the highest air temperatures. Mean temperature was highest in the thoracic cavity, at 33.3°C (Figure 5).

Table 3. Summary of decomposition trials.

Variable	Trial				Total
	1	2	3	4	
Location	Warra	Warra	Biloela	Biloela	
Start date	28/11/2023	22/1/2024	19/2/2024	14/3/2024	
End date	13/12/2023	3/2/2024	4/3/2024	2/4/2024	
Duration of trial (days)	14.9	12.1	13.2	18.2	
Number of pigs Total (M/F)[a]	8 (5/3)	8 (4/4)	8 (8/0)	8 (8/0)	
Mean weight (kg) (SD)[b]	51.5 (8.2)	51.5 (8.5)	48.8 (10.5)	67.5 (18.3)	
Total no. timepoints	17,136	13,920	15,168	20,976	67,200
No. of temp. recordings	13,816	10,299	13,595	18,845	56,555
No. hours without temp. readings (%)	19.4	26	10.4	10.2	
Temp. outliers (%)	12.7	13.2	13	14	
No. temp. recordings after outlier removal	12,059	8,942	11,831	16,207	49,039
No. pH recordings	13,693	10,089	13,580	18,749	56,111
No. hours without pH readings (%)	20.1	27.5	10.5	10.6	
pH outliers (%)	29.5	30	32.7	34.8	
No. pH recordings after outlier removal	9,651	7,065	9,146	12,221	38,083

[a] Male/female.
[b] Standard deviation.

Table 4. Summary statistics of ambient weather conditions across all trials obtained from the Australian Bureau of Meteorology weather stations located at Dalby Airport (Trials 1 & 2) and Thangool Airport (Trials 3 & 4).

Condition	Trial 1	Trial 2	Trial 3	Trial 4
Mean temperature °C (SE)[a]	25.0 (5.3)	26.9 (4.5)	26.2 (4.7)	24.1 (4.1)
Minimum temperature °C	14.1	18.4	15.9	15.5
Maximum temperature °C	35.9	37.4	35	34.5
Mean relative humidity % (SE)[a]	60.3 (22.8)	71.9 (17.2)	70.3 (22.5)	75.2 (19.0)
Minimum relative humidity %	22	30.5	28	27
Maximum relative humidity %	96	98	100	100
Mean daily rainfall mm (SE)[a]	0.0 (0.3)	2.6 (11.1)	1.1 (4.9)	0.9 (2.3)
Minimum daily rainfall mm	0	0	0	0
Maximum daily rainfall mm	5	73.2	29.2	12.2
Total rainfall mm	16.3	818.7	387	433.5
Hours of recorded rain	21	48	39	105

[a] Standard error.

Mean temperature at other anatomical sites ranged from 32.0°C to 32.9°C. Differences between anatomical sites were statistically significant (ANOVA $P < 0.0001$); however, this difference was not considered biologically meaningful (<1.3°C).

The relationship between pH and anatomical site was modelled using a multivariable GAM approach, which revealed complex and strongly nonlinear relationships (Table 5). Model fit was reasonably good, with an R^2 value of 69%. The model revealed significant differences in pH between all anatomical sites, except between superficial muscle and thoracic cavity, indicating that different anatomical sites experience different post-mortem pH changes when considering multiple predictor variables. In contrast, when multiple predictors are not considered (i.e. in Figure 3), these differences may be overlooked. For example, differences between brain and superficial muscle are only apparent when accounting for mixed effects.

Given the relationship between air temperature and carcass temperature was largely linear (Figure 6), the relationships between temperature and anatomical sites were modelled using mixed effects multivariable linear regression. Model fit was reasonably good, with an R^2 value also of 69%. There were significant differences in temperature between all anatomical sites when accounting for multiple predictors (Table 6).

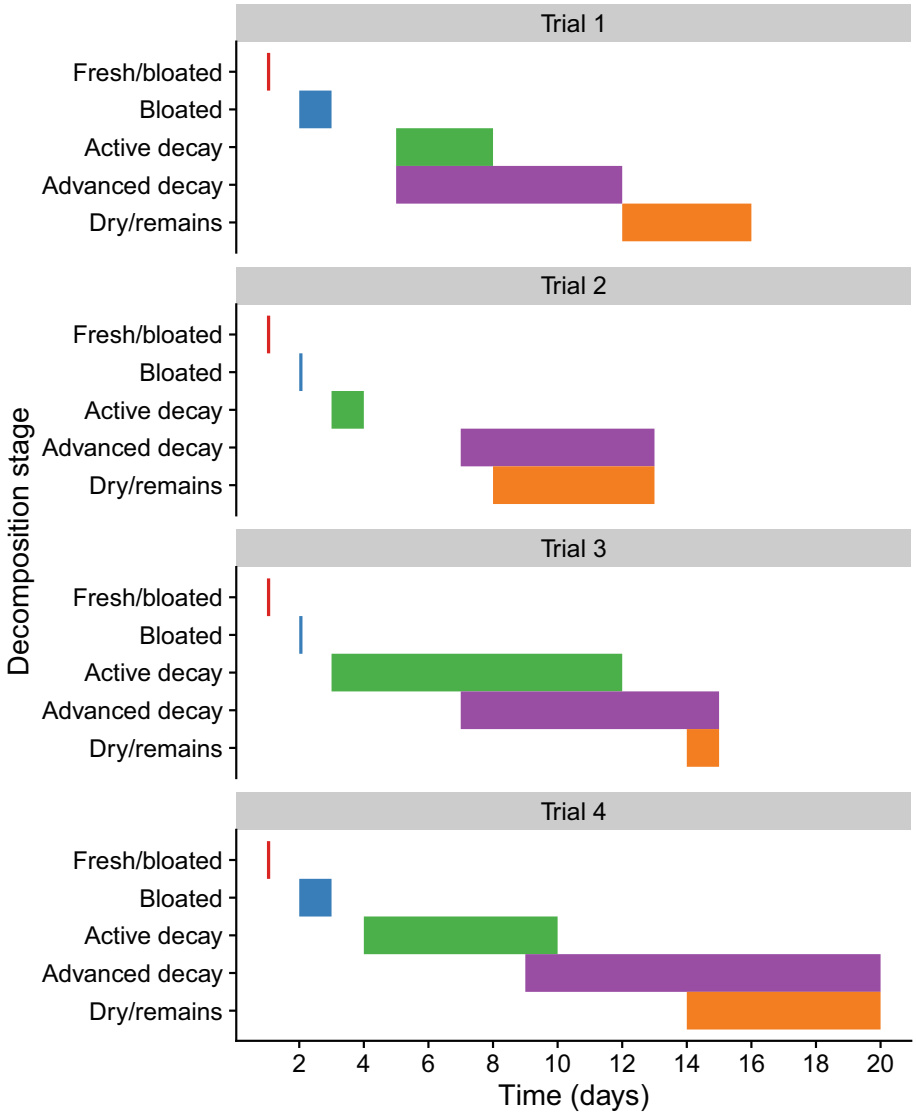

Figure 1. **Decomposition of pig carcasses left *in situ*. Pig carcasses (n = 8 per trial) were monitored visually every 1–2 days, and decomposition was classified into five stages. Trials 1 and 2 were conducted in the Western Downs region of southern inland Queensland, whereas trials 3 and 4 were conducted in central Queensland. Decomposition did not proceed uniformly in all pigs, resulting in overlapping stages. The duration of the final stage reflects the timing of sensor removal, not the natural progression of decomposition. Gaps indicate days when carcasses were not inspected.**

Factors influencing carcass pH and temperature

Using a GAM and a machine learning-assisted RF algorithm, the most important variable influencing carcass pH in both analyses was days since humane killing, followed by anatomical site (Figure 7 and Table 5). Additionally, air temperature, rainfall, relative humidity, study location and animal identifier were all highly significant ($P < 0.0001$) (Table 5).

For carcass temperature, both linear mixed effects modelling and a machine learning algorithm identified that the most important predictor was air temperature, with other important variables including days since humane killing and rainfall (Figure 8 and Table 6). Additionally, the RF model identified humidity as an important predictor of carcass temperature, which was not included in the linear mixed effects model due to collinearity with air temperature (Figure 8). However, this collinearity could be handled by an RF model. Furthermore, study location was also found to significantly affect carcass temperature ($P < 0.001$). Temperature was, on average, 1.01°C higher at Warra compared with Biloela (Table 6).

We used the RF models to predict pH at each anatomical site for 14 days post humane killing and temperature at each anatomical site across an ambient temperature range of 14°C–37°C (i.e. within the scope of the model) (Supporting Information). These predictions may be used to inform future risk assessments around the suitability of the D&LL carcass disposal method depending on the environmental conditions at the culling location.

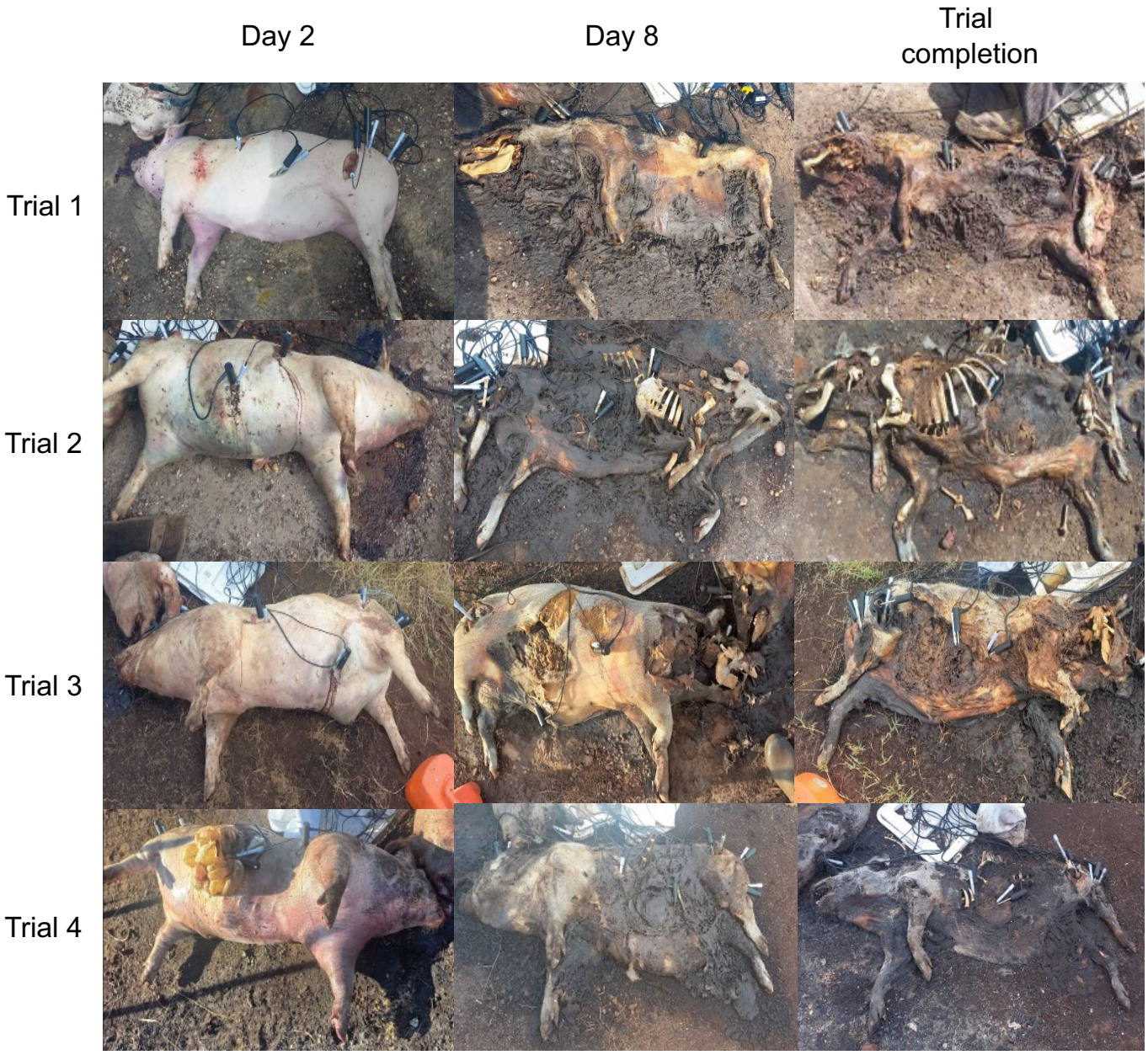

Figure 2. **Decomposition of pig carcasses left *in situ* on day 2, day 8 and at trial completion. Pig carcasses (n = 8 per trial) were monitored visually every 1–2 days. Trials 1 and 2 were conducted in the Western Downs region of southern inland Queensland, whereas trials 3 and 4 were conducted in central Queensland. Trial durations were 14.9, 12.1, 13.2 and 18.2 days, respectively. Note that the images are not of the same pig carcass in each trial.**

To investigate the potential effects of internal organ disruption on carcass decomposition, three carcasses per trial (n = 12) received a post-mortem abdominal shot in addition to a head shot. The remaining 20 carcasses received only a head shot. The effect of an abdominal shot was not statistically significant for either carcass pH or temperature based on our statistical models (pH P = 0.268; temperature P = 0.278).

Inferences around ASFV and FMDV inactivation

Throughout all trials, no pH or temperature values at any anatomical site exceeded the upper or lower ASFV inactivation threshold across three or more pigs (Figures 9 and 10). One pig showed an abdominal pH reading of <6 at the end of the trial, which was determined to be an outlier that was not captured in our automated data cleaning methodology. A threshold of 56°C for ≥2 hours was observed in the bone marrow of one pig carcass in trial 3; however, no other pigs recorded similar readings.

For FMDV, pH inactivation thresholds were reached in some anatomical sites (Figures 11 and Supporting Information). Throughout all trials, the lower (acidic) pH threshold was consistently reached by most sensors in the abdominal cavity, deep muscle, superficial muscle and thoracic cavity (Supporting Information). The lower threshold was generally reached in the first 14 hours of the trials, after the

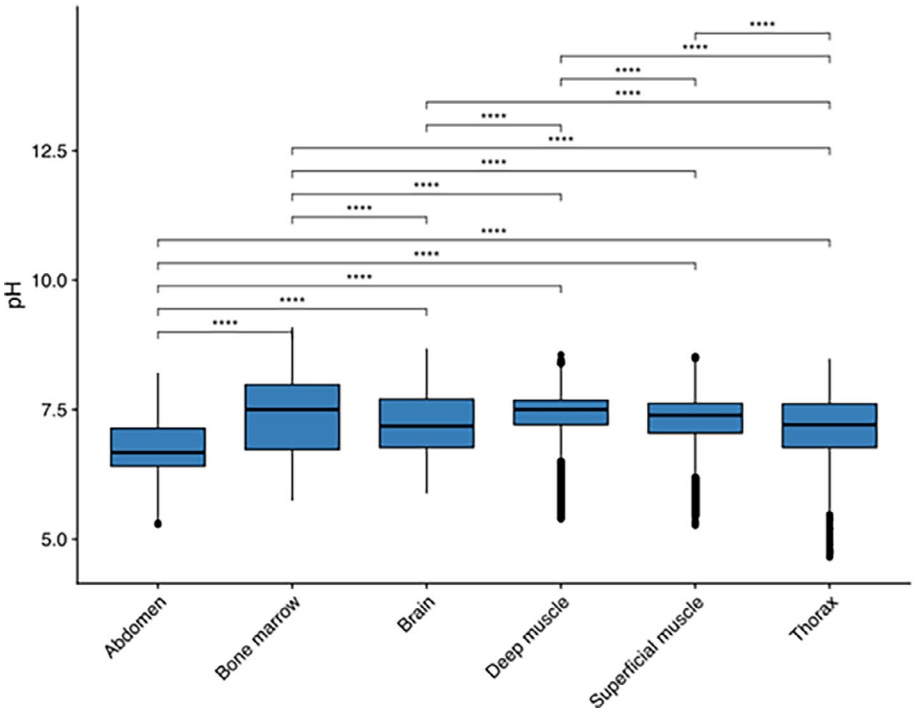

Figure 3. Tissue pH by anatomical site. Shown are boxplots of the pH measurements collected hourly from each pig across all four trials, split by anatomical site. In contrast to mixed effects modelling, this does not capture effects due to other predictors. The central boxes show the 25th, 50th (median) and 75th percentiles. Whiskers represent 1.5× the interquartile range above and below quartile 1 and quartile 3. Data points outside this range are shown as outliers. Significance was determined using Tukey's HSD test (****$P < 0.0001$).

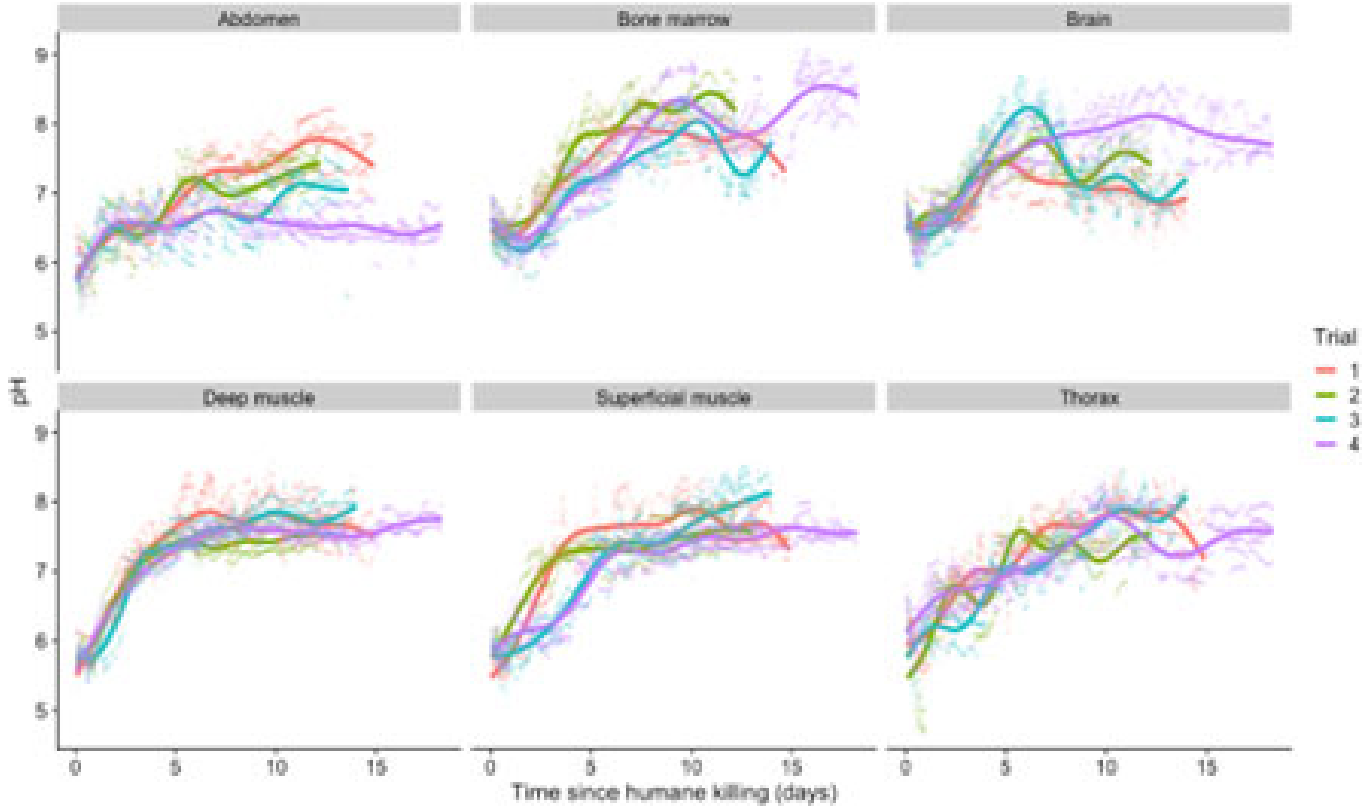

Figure 4. Tissue pH as a function of anatomical site and time since humane killing. Dots represent individual observations colour-coded by trial. A line of best fit was fitted as a univariable generalised additive model for each trial and anatomical site. Plots are faceted by anatomical site. Data were standardised across trials by calculating the time since humane killing in days (x-axis).

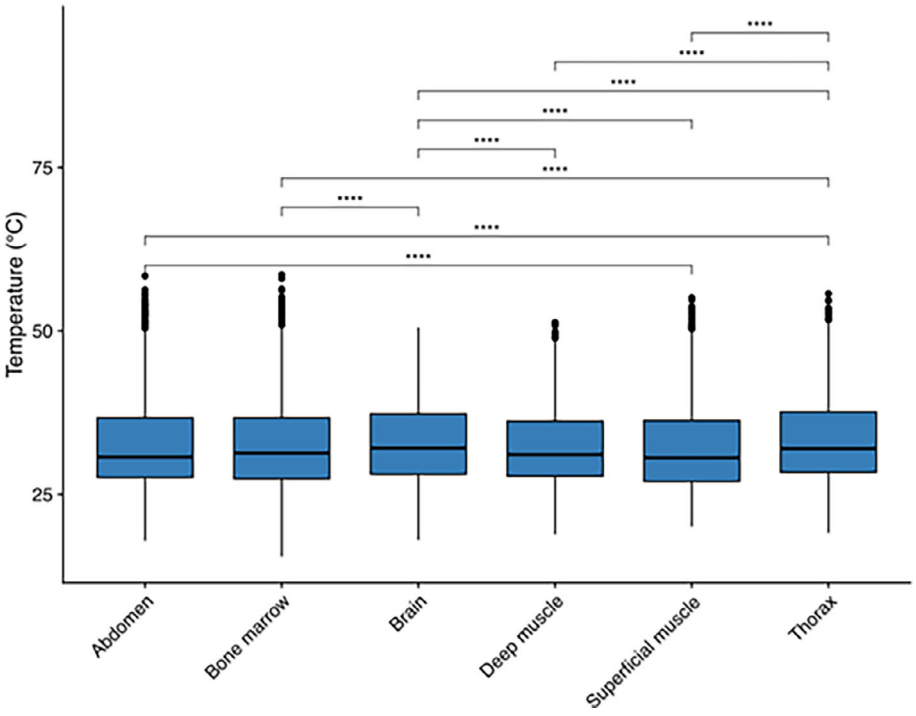

Figure 5. Tissue temperature by anatomical site. Shown are boxplots of temperature measurements collected hourly from each pig across all four trials, split by anatomical site. In contrast to mixed effects modelling, this does not capture effects due to other predictors. The central boxes show the 25th, 50th (median) and 75th percentiles. Whiskers represent 1.5× the interquartile range above and below quartile 1 and quartile 3. Data points outside this range are shown as outliers. Significance was determined using Tukey's HSD test; only significant differences ($P < 0.05$) of at least 2°C are shown (****$P < 0.0001$).

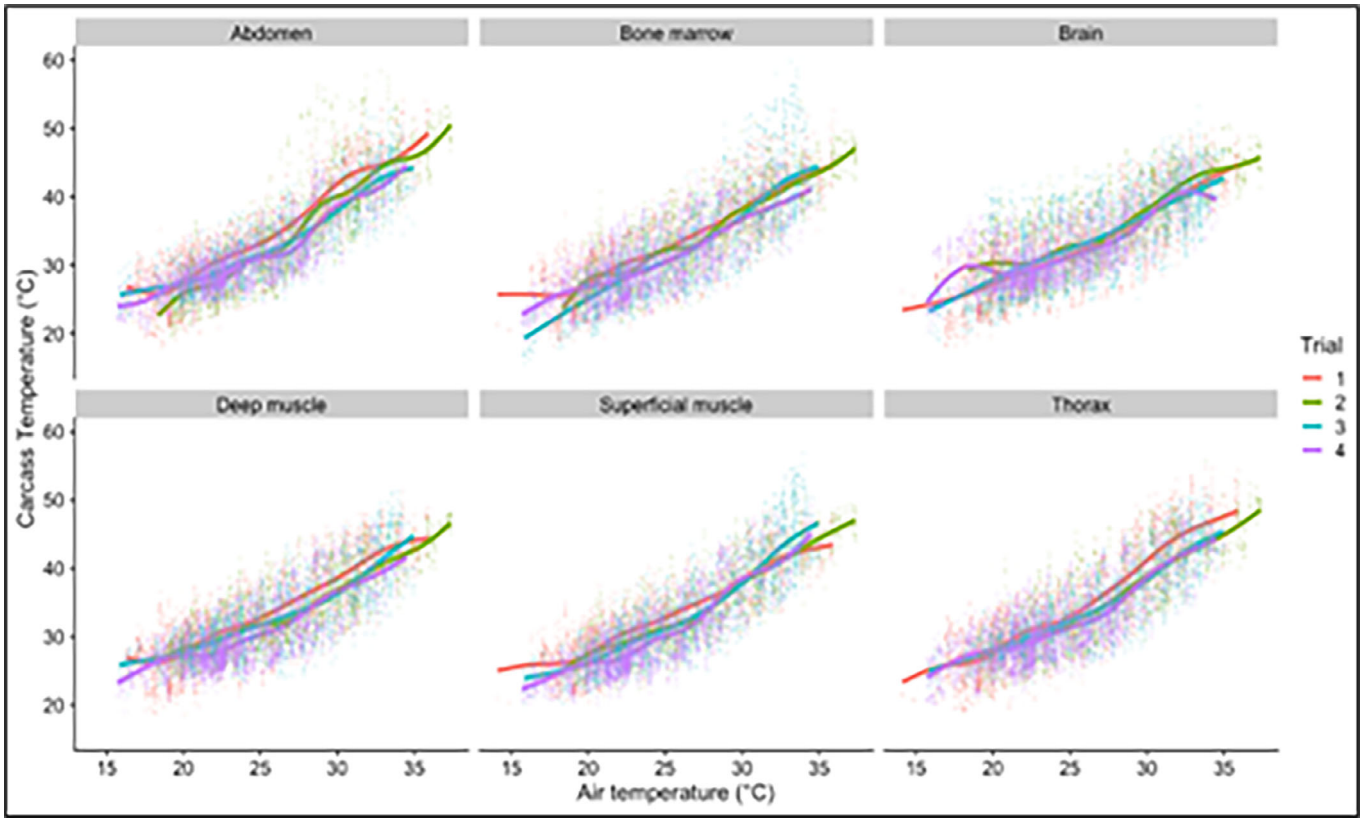

Figure 6. Tissue temperature as a function of air temperature. Dots represent individual observations colour-coded by trial. A line of best fit was fitted using a univariable generalised additive model for each trial and anatomical site. Plots are faceted by anatomical site.

Table 5. Model results for the pH GAM fitted to estimate carcass pH from anatomical site while adjusting for other relevant variables.

Parametric coefficients	Estimate	Standard error	t-value	P-value
(intercept)	6.709	0.068	98.603	<0.001
Sex (female)	Reference			
Sex (male)	0.005	0.061	0.076	0.940
Abdominal cavity	Reference			
Bone marrow	0.723	0.007	106.317	<0.001
Brain	0.543	0.007	80.970	<0.001
Deep muscle	0.489	0.007	71.459	<0.001
Superficial muscle	0.388	0.007	56.175	<0.001
Thoracic cavity	0.390	0.007	57.913	<0.001
Study location (Biloela)	*Reference*			
Study location (Warra)	0.129	0.050	2.561	0.011

Approximate significance of smooth terms	Smoothing df[a]	Reference df[a]	F-value	P-value
s(days since humane killing)	8.933	8.998	7206.394	<0.001
s(air temperature)	4.138	5.190	6.244	<0.001
s(rainfall)	7.723	8.227	7.750	<0.001
s(animal identifier)	28.731	29.000	110.980	<0.001
s(humidity)	8.341	8.874	12.035	<0.001

Measures of model fit. Adjusted $R^2 = 0.688$. Deviance explained = 68.9%. Minimised generalised cross-validation score = 0.136. Scale estimate = 0.136. Number of observations = 38,035.
[a] Degrees of freedom.

Table 6. Model results from the linear mixed effects model fitted to estimate carcass temperature from anatomical site while adjusting for other relevant variables.

Parametric coefficients	Estimate	Standard error	t-value	P-value
(intercept)	5.065	0.296	17.119	<0.001
Days since humane killing	−0.039	0.004	−10.426	<0.001
Air temperature	1.098	0.004	311.328	<0.001
Rainfall	−0.065	0.003	−23.962	<0.001
Sex (female)	*Reference*			
Sex (male)	0.062	0.248	0.248	0.806
Abdominal cavity	*Reference*			
Bone marrow	−0.842	0.056	−15.032	<0.001
Brain	−0.230	0.055	−4.155	<0.001
Deep muscle	−0.651	0.056	−11.686	<0.001
Superficial muscle	−0.660	0.057	−11.513	<0.001
Thoracic cavity	0.475	0.056	8.520	<0.001
Study location (Biloela)	*Reference*			
Study location (Warra)	1.015	0.205	4.943	<0.001
Random effects				
Name	Variance	Standard deviation		
Animal identifier	0.233	0.483		
Residual	12.580	3.547		
Model fit				

Conditional $R^2 = 0.697$. Marginal $R^2 = 0.692$, Number of observations = 48,986. Groups = Animal identifier (32). Animal identifier categorical predictors have been excluded for simplicity.

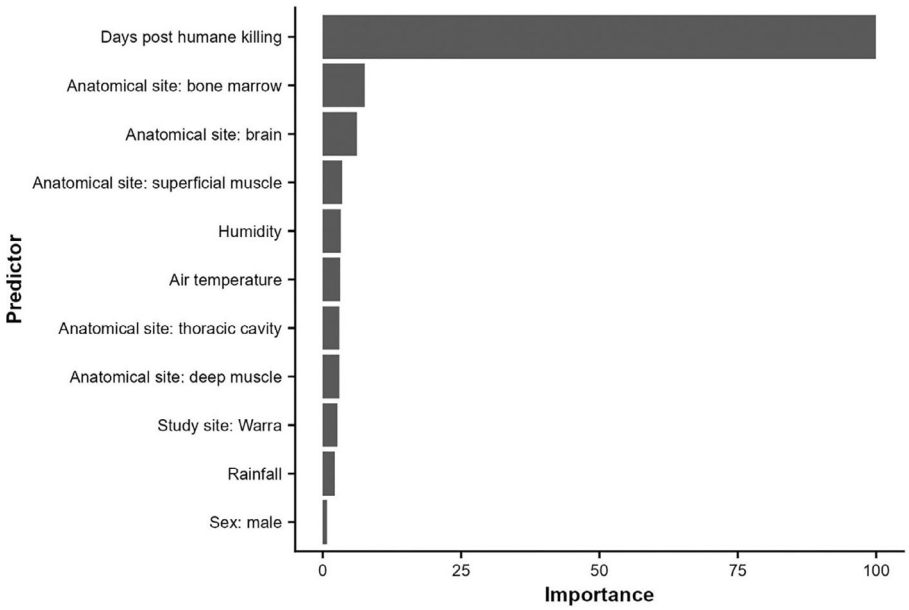

Figure 7. Variable importance plot for the random forest (RF) machine learning algorithm to predict carcass pH. A RF model was fitted and trained to explore relationships between carcass pH and various predictor variables. A relative index of the importance of each predictor is shown as a bar. The detailed methodology for the calculation of this relative index is given in the documentation for the importance function within the randomForest R package.[52]

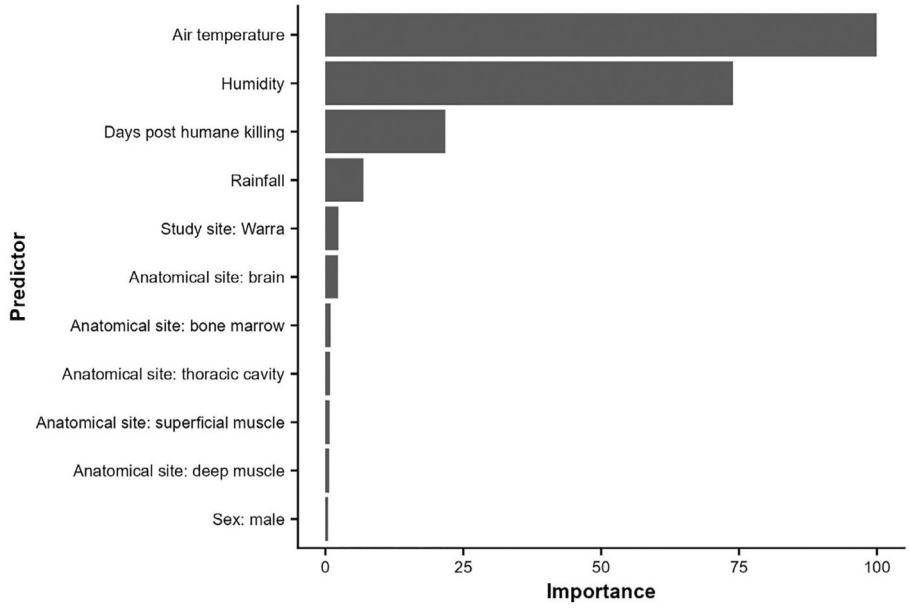

Figure 8. Variable importance plot for the random forest (RF) machine learning algorithm to predict carcass temperature. A RF model was fitted and trained to explore relationships between carcass temperature and various predictor variables. A relative index of the importance of each predictor is shown as a bar. The detailed methodology for the calculation of this relative index is given in the documentation for the randomForest::importance function.[52]

expected drop in pH post humane killing. The time to reach the lower pH inactivation threshold was fastest in deep muscle, with a median time of 2 hours, followed by superficial muscle and thorax, which reached the threshold within a combined median time of 7 hours. Only one pig (trial 3) met the lower pH inactivation threshold in all six anatomical sites (Supporting Information). The upper (alkaline) FMDV pH inactivation threshold was recorded for two sensors in bone marrow late in trial 4. These are unlikely to be true readings, as bone marrow by this stage had typically dried out. FMDV temperature inactivation thresholds were occasionally reached, but less reliably than pH thresholds (Figures 12 and Supporting Information). No pigs met FMDV temperature inactivation thresholds across all six anatomical sites (Supporting Information).

When considering both the pH and temperature inactivation thresholds together, two pigs had recordings consistent with FMDV inactivation in all six anatomical sites (Supporting Information). A further three pigs met FMDV inactivation thresholds in all sites where sensors remained active (i.e. some probes did not record, but remaining probes all met inactivation thresholds). For the remaining 27 pigs (84%) in this study, FMDV inactivation criteria were not met in one or more anatomical sites.

Discussion

This study explored decomposition dynamics, including internal pig carcass temperature and pH changes, during natural above-ground

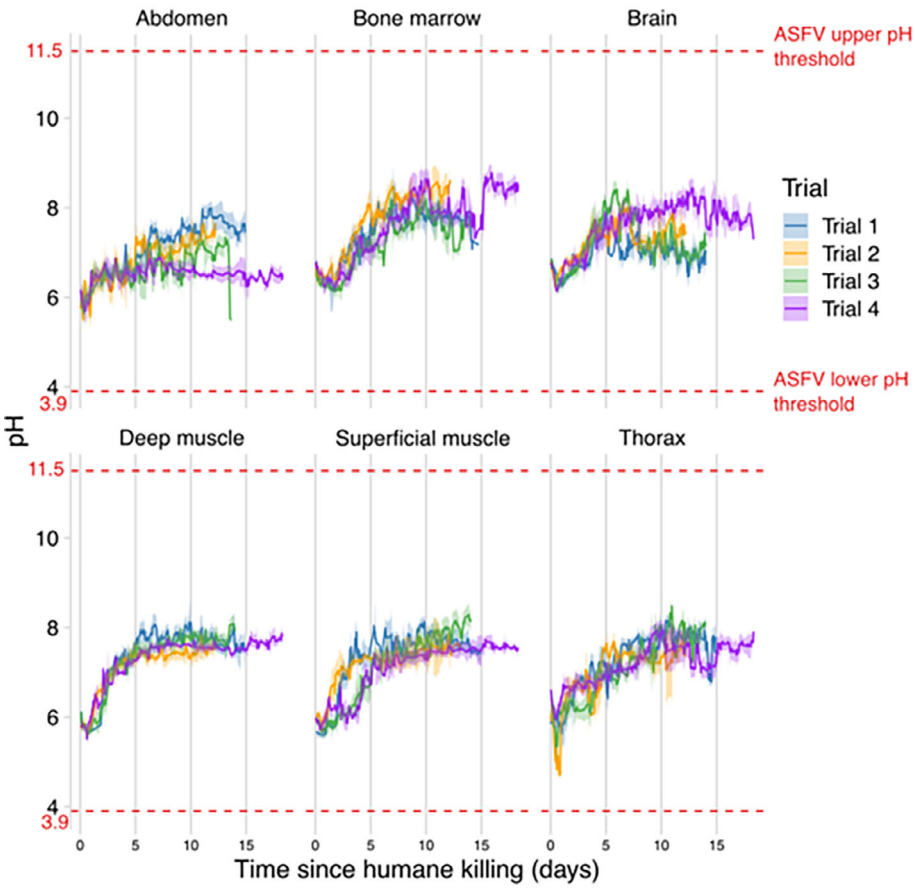

Figure 9. Potential for ASFV inactivation through pH effects. pH over time by anatomical site is shown. Lines represent the mean of all probes at each timepoint and are colored by trial. Ribbons show the 95% confidence interval around the mean. Plots are faceted by anatomical site. Data were standardised across trials by calculating the time since humane killing in days (x-axis). ASFV inactivation thresholds are marked by dashed red lines and labelled.[39,40]

decomposition under Queensland conditions to inform whether pathogens could be inactivated, based on carcass pH and temperature, using a D&LL disposal approach. This work has expanded upon a previous study (Stage 1) by exploring carcass changes beyond 48 hours.[9]

Time to end-stage decomposition ranged from 12.1 to 18.2 days, with trial 4 having the longest duration. The size of the pigs used in trial 4 likely influenced the rate of decomposition. The average weight of feral pigs in Australia ranges from 50 to 60 kg for females, up to 100 kg for males.[25] Therefore, the generalisability of our findings to large feral boars may be limited. Other pig studies have found that decomposition can take up to 30 days without the presence of scavengers, or as little as 7–10 days with scavenger and insect activity.[26–28]

We defined end-stage decomposition based on the state of soft tissues. Carcass remains, including bone, skin and leached soluble body contents, were still present (Figure 2). In the context of the application of the D&LL disposal method, infectious virions may remain in or on tissue fragments (e.g. bone) and may leach into the surrounding soil and ground/surface water as a carcass decomposes.[29,30] Scavengers such as birds, rodents and other mammals may disperse the virus away from the initial carcass site, either becoming contaminated with bodily fluids while feeding or through the transport of infectious body parts.[31,32]

Significant declines in pH were observed primarily within the first 48 hours, before trending towards neutral for the remainder of the trials. In agreement with the observations, the most important predictor for carcass pH was days since humane killing. This aligns with the trends seen in previous studies exploring pig decomposition *in situ*.[9] The observed pH changes were not as marked as those in the previous Stage 1 D&LL study, with pH values up to 1.1 pH units higher in our study. This difference could be due to several factors. Stage 1 used both feral and domestic pigs, whereas we used domestic pigs only. Carcass pH profiles are known to differ between breeds.[33] Furthermore, different handling and destruction methods were implemented between the studies.

The highest carcass temperatures were associated with elevated air temperatures above 32°C. Carcasses will typically trend towards the ambient temperature after humane killing and will likely be influenced by factors such as carcass temperature at the time of death, composition of the carcass, for example, size, mass, muscle and fat composition, and other insulating factors such as coat type and colour.[34,35] Shortly after death, internal temperatures will reduce with algor mortis and then increase in the bloated stage, associated with putrefaction and the metabolic activities of maggot masses.[36] For example, larval masses of the blowfly *Lucilia sericata* can increase carcass temperature by 2.5–14°C,[37] although this temperature increase is typically localised to body cavities.[38] This may have influenced the elevated temperatures seen in the abdomen. Larval masses and their secretions generally tend towards a neutral to alkaline pH ,[27] consistent with the findings in our study where acidic tissue pH was not maintained.

The most important predictor of carcass temperature was air temperature, with other notable predictors including humidity, days

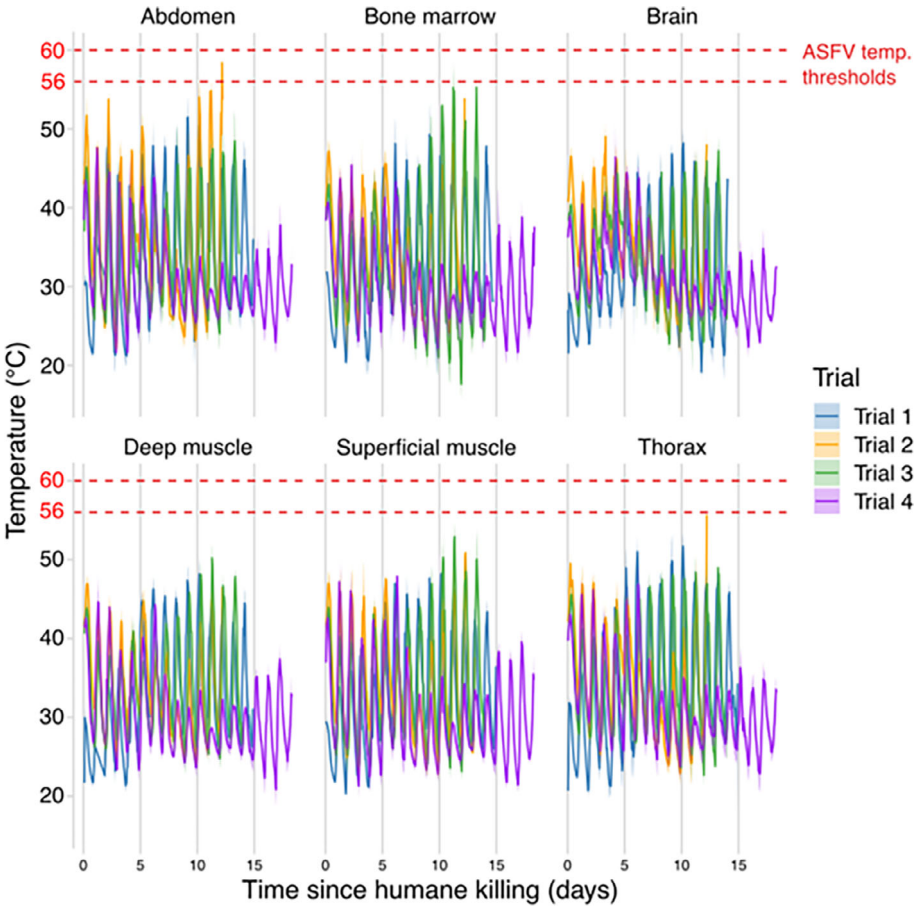

Figure 10. **Potential for ASFV inactivation through temperature effects. Temperature over time by anatomical site is shown. Lines represent the mean of all probes at each time point and are coloured by trial. Ribbons show the 95% confidence interval around the mean. Plots are faceted by anatomical site. Data were standardised across trials by calculating the time since humane killing in days (x-axis). ASFV inactivation thresholds are marked by dashed red lines and labelled.[39,41]**

since humane killing and rainfall. This suggests that carcass temperature is unlikely to exceed temperature thresholds required for FMDV and ASFV in more southern areas of Australia or where extreme, higher temperatures are less likely. Interestingly, for both carcass temperature and pH, we observed an effect of geographic location when controlling for environmental conditions and other predictors. This suggests that carcass temperature and pH are influenced by one or more unmeasured environmental variables that differ between study locations.

Although this study did not involve any primary virological experiments, the temperature and pH conditions observed in the pig carcasses were not conducive to inactivating ASFV, based on widely accepted temperature and pH inactivation thresholds.[39–41] Taking the effects of temperature and pH together, FMDV was likely to be inactivated in the thoracic cavity, superficial and deep skeletal muscle and abdominal cavity of most carcasses.[8,42] FMDV was less likely to be inactivated in the bone marrow and was least likely to be inactivated in the brain. We found that post-mortem temperature and pH dynamics vary significantly between anatomical sites, highlighting the importance of investigating multiple tissues. Critically, there are other tissues that were not examined that are of high relevance for ASFV and/or FMDV transmission. For example, ASFV loads are highest in the bone marrow, lymph nodes and blood[43] and FMDV viral loads are typically highest in epithelium but are also high in blood, bone marrow and lymph nodes.[33] Tissues such as lymph nodes undergo little to no post-mortem acidification due to compartmentalisation from muscle through connective tissue layers.[30,44–46] When considering disposal in an EAD response, the unit of interest is the carcass. Therefore, virus must be inactivated in all tissues to render a carcass noninfectious. However, the absolute viral load present in the environment will be reduced if fewer tissues remain infective.

Our findings suggest that ≥84% of ASFV- or FMDV-infected carcasses may have remained infectious under the environmental conditions experienced during the trials, based on the inactivation thresholds used. Importantly, viral inactivation is more complex than simple pH and temperature threshold criteria. Several previous studies have shown that there are insufficient primary virological data available in the literature to robustly estimate ASFV and FMDV decay rates in different tissues, particularly under nonconstant conditions.[47,48] Although numerous studies have explored ASFV and FMDV survivability in tissues, most of these studies were conducted in controlled environments under constant conditions, with very few examining survival in natural field conditions with fluctuations in experimental conditions. Critically, this

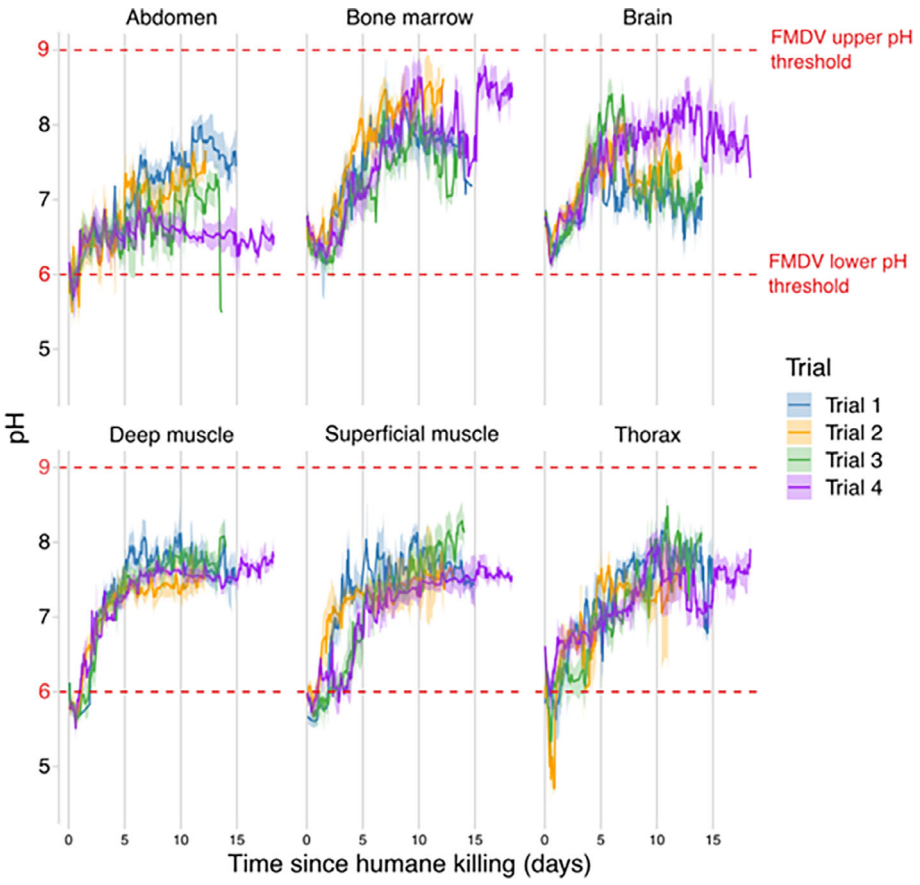

Figure 11. Potential for FMDV inactivation through pH effects. pH over time by anatomical site is shown. Lines represent the mean of all probes at each time point and are coloured by trial. Ribbons show the 95% confidence interval around the mean. Plots are faceted by anatomical site. Data were standardised across trials by calculating the time since humane killing in days (x-axis). FMDV inactivation thresholds are marked by dashed red lines and labelled.[42]

study, along with previous D&LL research,[9] has determined the temperature and pH dynamics that a pig carcass may experience under southeast and central Queensland environmental conditions during late summer and autumn. Knowing this information, primary virological studies (e.g. with surrogate viruses) can be conducted within these temperature and pH ranges to robustly understand viral inactivation under these conditions.

Infectious disease transmission is complex and stochastic, depending on the proportion of susceptible animals in a population, the contact rate between susceptible animals and infectious material, and the probability of infection given contact (which depends on factors including the amount of infectious virus present/transferred and the minimum infective dose).[49,50] Although infected pig carcasses may remain infectious after culling in the field, this may be of little epidemiological consequence during an outbreak in Australia. Repeated and independent modelling has indicated that the culling of feral pigs in a disease-affected area of Australia could lead to disease fadeout in the Australian environment, whether or not carcasses are considered.[4–6,51] This is in contrast to the European context, where carcasses are believed to have an important epidemiological role in ASF maintenance.[2] These models all indicated that the culling of the population will lead to reduced contact and hence reduced transmission. In addition, if culling is spatially large enough, the culled area will pass the edge of the infected zone, and populations around the infected areas will be depressed, reducing the chance of transmission outside an infected area. When assessing the suitability of D&LL for carcass disposal, a risk assessment considering the efficacy of culling tools in a location, potential for disease spread by predators and scavengers and the potential for introduction into epidemiologically distinct populations to continue transmission should be conducted.[7] This should be balanced against the risks, benefits and feasibility of alternative disposal methods.

Several limitations of this study must be noted. The predictive power of the models developed is limited to the scope of the experimental data. Although overfitting is a concern in all models, our analysis approach was designed to mitigate this risk. Regression models were used for exploration rather than prediction, whereas RF models were chosen for their resilience to overfitting. As such, predictions around carcass temperature and pH should be inferred from the RF models. Through techniques such as cross-validation, ensemble averaging, hyperparameter tuning and using robust evaluation metrics like mean absolute error, we ensured that our models were robust and avoided overfitting. The environmental conditions (i.e. air temperature, relative humidity, rainfall) were obtained from the Bureau of Meteorology and were measured at a location some distance from the study location; exposed carcasses may have experienced slightly different ambient conditions. Throughout the course of the study, we experienced temperature and/or pH sensor malfunctions at various times. Sensors were checked at least every 2 days, pH sensors were calibrated every 5 days, and obvious outliers were excluded during data cleaning; however, missing data may have limited the precision of our results. Bone marrow access required sectioning of the femur with exposure of bone marrow to the ambient environment. This would have led to more rapid degradation and

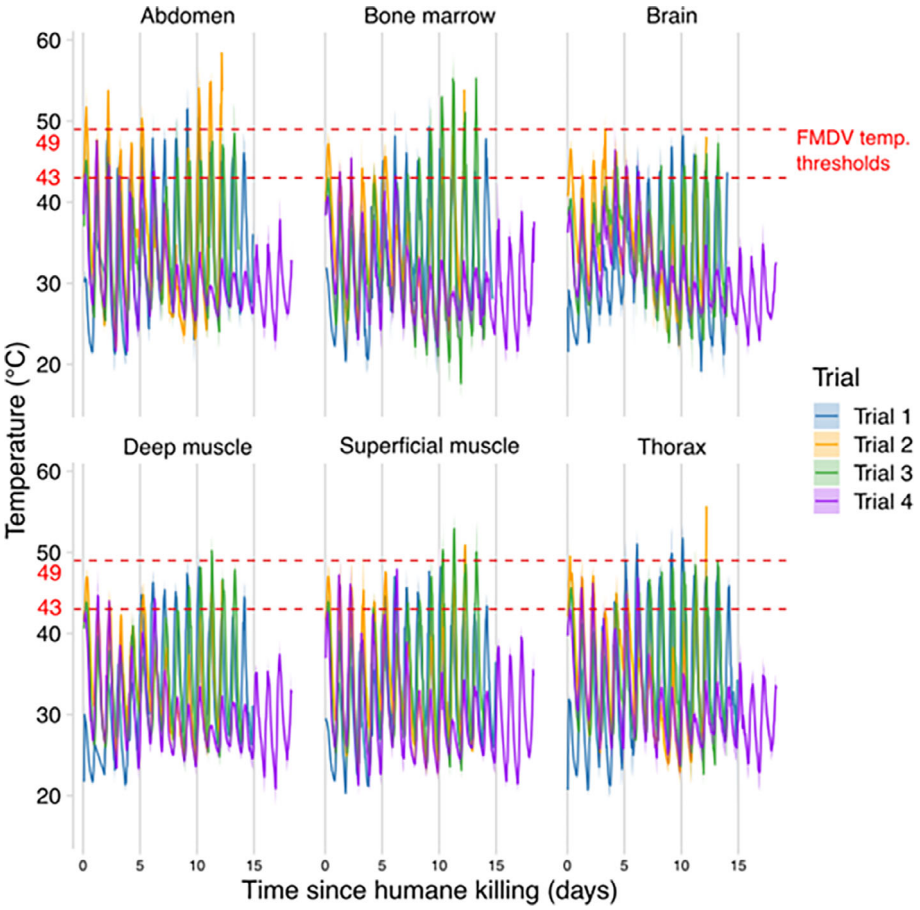

Figure 12. **Potential for FMDV inactivation through temperature effects. Temperature over time by anatomical site is shown. Lines represent the mean of all probes at each time point and are coloured by trial. Ribbons show the 95% confidence interval around the mean. Plots are faceted by anatomical site. Data were standardised across trials by calculating the time since humane killing in days (x-axis). FMDV inactivation thresholds are marked by dashed red lines and labelled.[8]**

breakdown of tissue, not representative of marrow sealed within a bone. To the best of our abilities, the surrounding muscle was placed for protection over the incision. However, tissues rapidly broke down after death, and the bone was exposed for most of the trial. Similarly, for the brain, the process of humane killing involved a captive bolt and head shot, which resulted in exposure of brain tissue to the environment and maggots, and relatively rapid tissue degradation. Domestic pig carcasses were used for this study, whereas the D&LL methodology is most likely to be applied to feral pigs. The finding that geographical location significantly affected carcass temperature and pH indicates that similar trials must be carried out in additional geographical areas, particularly those where D&LL would potentially be employed if found suitable. Finally, the carcasses were protected from almost all scavengers. In reality, carcass removal from the environment may be much more rapid than recorded here, reducing viral persistence in the environment, although this may also lead to dissemination of potentially infectious material.[32]

The use of D&LL as a management technique to achieve the objective of complete viral inactivation within an infected carcass under northern Australian conditions cannot be applied with confidence based upon our findings. Although many selected anatomical sites did reach an inactivation threshold, indicating a reduction in carcass infectivity was likely, there was almost always at least one anatomical site that did not reach the inactivation threshold in each carcass, indicating that viral inactivation may not occur based on pH and temperature parameters. In addition, we did not investigate other anatomical sites and tissues which are often more infectious, particularly where viral persistence is likely to occur, for example, lymph nodes and blood clots. Primary viral inactivation studies under the observed carcass pH and temperature conditions are required for enhanced confidence in whether carcasses would remain infectious. The D&LL disposal approach, however, may still support disease control imperatives during an EAD response, mitigating logistical challenges in the management of feral pig carcasses and suppressing virus transmission by reducing live, susceptible pig numbers. The specific disposal method/s adopted during an EAD response will need to be considered in the context of the outbreak, location and environment.

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Conflicts of interest and sources of funding

RNH, OS, JT, AH, EECL and BDC are employed by the company Ausvet Pty Ltd. TSB is a working director of Epivet Pty Ltd. KR is employed by the company SunPork Group. None of the authors have financial or personal interests that could influence or bias the content of the manuscript. A scientific steering committee, comprising members of the funding agency and individuals who have an interest in the outcome of the work, provided input into the commissioning, conception, planning, design and conduct of the work and the decision to publish.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Hone J. How many feral pigs in Australia? *An update Aust J Zool* 2020;67:215–220. https://doi.org/10.1071/ZO20077.
2. Sauter-Louis C, Conraths FJ, Probst C et al. African swine fever in wild boar in europe—a review. *Viruses* 2021;13:1717. https://doi.org/10.3390/v13091717.
3. Mohamed F, Swafford S, Petrowski H et al. Foot-and-mouth disease in feral swine: susceptibility and transmission. *Transbound Emerg Dis* 2011;58:358–371. https://doi.org/10.1111/j.1865-1682.2011.01213.x.
4. Cowled BD, Garner MG, Negus K et al. Controlling disease outbreaks in wildlife using limited culling: modelling classical swine fever incursions in wild pigs in Australia. *Vet Res* 2012;43:3. https://doi.org/10.1186/1297-9716-43-3.
5. Bradhurst R, Roche S, Garner G et al. *Modelling the spread and control of African swine fever in domestic and feral pigs*. Melbourne, Australia, Centre for Excellence for Biosecurity Risk Analysis, 2021 Report No.: CEBRA Project 20121501. https://cebra.unimelb.edu.au/__data/assets/pdf_file/0003/3949500/CEBRA_project_20121501_final_report_1.4.pdf.
6. Ward MP, Garner MG, Cowled BD. Modelling foot-and-mouth disease transmission in a wild pig–domestic cattle ecosystem. *Aust Vet J* 2015;93:4–12. https://doi.org/10.1111/avj.12278.
7. Animal Health Australia. *Operational manual: disposal (version 5.0)*. Canberra, ACT, Australian Veterinary Emergency Plan (AUSVETPLAN), 2021 https://animalhealthaustralia.com.au//wp-content/uploads/dlm_uploads/2021/12/AUSVETPLAN-_Operational_Disposal_Manual.pdf.
8. Hunnam J, Duff K, Wingett M et al. Effect of carcase decomposition on the inactivation of foot-and-mouth disease virus under northern Australian conditions. *Aust Vet J* 2018;96:332–340. https://doi.org/10.1111/avj.12731.
9. Cobbold RN, Barnes TS, Moore TA. *Carcass disposal – destroy and let lie (D&LL) research interpretive report*. Gatton, Queensland, Australia, The University of Queensland, 2023 Report No.: eDOCS #18402305.
10. DEEDI, DAFF. Destroy and let lie project report: a project undertaken as part of the wildlife exotic disease preparedness program. In: *Department of Employment, economic development and innovation, Department of Agriculture Fisheries & forestry*. Australia, Queensland Government, 2010.
11. Duc HM, Ngan PH, Son HM et al. The use of composting for the disposal of African swine fever virus-infected swine carcasses. *Transbound Emerg Dis* 2022;69:e3036–e3044. https://doi.org/10.1111/tbed.14659.
12. Duc HM, Hutchinson M, Flory GA et al. Viability of African swine fever virus with the shallow burial with carbon carcass disposal method. *Pathogens* 2023;12:628. https://doi.org/10.3390/pathogens12040628.
13. Ebling R, Paim WP, Turner J et al. Virus viability in spiked swine bone marrow tissue during above-ground burial method and under in vitro conditions. *Transbound Emerg Dis* 2022;69:2987–2995. https://doi.org/10.1111/tbed.14462.
14. Gabbert L, Martignette L, Zurita M et al. Evaluation of whole carcass composting as a mortality disposal option for African swine fever virus-infected swine. *Transbound Emerg Dis* 2023;2023:e9926250. https://doi.org/10.1155/2023/9926250.
15. Guan J, Chan M, Grenier C et al. Degradation of foot-and-mouth disease virus during composting of infected pig carcasses. *Can J Vet Res* 2010;74:40–44.
16. Hutchinson M, Duc HM, Flory GA et al. Static aerated composting of African swine fever virus-infected swine carcasses with rice hulls and sawdust. *Pathogens* 2023;12:721. https://doi.org/10.3390/pathogens12050721.
17. Merchioratto I, Mendes Peter C, Ramachandran A et al. Viability of veterinary-relevant viruses in decomposing tissues over a 90-day period using an in-vitro system. *Pathogens* 2023;12:1104. https://doi.org/10.3390/pathogens12091104.
18. Thaler B. *Above ground burial/composting of swine mortalities*. Brookings, South Dakota, South Dakota State University, 2021 Report No.: #19–218. https://www.porkcheckoff.org/wp-content/uploads/2022/05/19-218-IPPA-THALER-final-rpt.pdf.
19. Zani L, Masiulis M, Bušauskas P et al. African swine fever virus survival in buried wild boar carcasses. *Transbound Emerg Dis* 2020;67:2086–2092. https://doi.org/10.1111/tbed.13554.
20. Sharp T. *NATSOP-PIG002 national standard operating procedure: aerial shooting of feral pigs*. Australia, PestSmart website, 2012.
21. Payne JA. A summer carrion study of the baby pig sus scrofa Linnaeus. *Ecology* 1965;46:592–602. https://doi.org/10.2307/1934999.
22. Buskirk TD. Surveying the forests and sampling the trees: an overview of classification and regression trees and random forests with applications in survey research. *Surv Pract* 2018;11:1–13. https://doi.org/10.29115/SP-2018-0003.
23. James G, Witten D, Hastie T et al. *An introduction to statistical learning: with applications in R*. 2nd edition. New York, NY, Springer US, 2021. https://doi.org/10.1007/978-1-0716-1418-1.
24. Voss SC, Spafford H, Dadour IR. Annual and seasonal patterns of insect succession on decomposing remains at two locations in Western Australia. *Forensic Sci Int* 2009;193:26–36. https://doi.org/10.1016/j.forsciint.2009.08.014.
25. Centre for Invasive Species Solutions. *Feral pig biology, ecology and behaviour*. Canberra, Australia, PestSmart, 2011.
26. Griffiths K, Krosch MN, Wright K. Variation in decomposition stages and carrion insect succession in a dry tropical climate and its effect on estimating postmortem interval. *Forensic Sci Res* 2020;5:327–335. https://doi.org/10.1080/20961790.2020.1733830.
27. Junkins EN, Speck M, Carter DO. The microbiology, pH, and oxidation reduction potential of larval masses in decomposing carcasses on Oahu, Hawaii. *J Forensic Leg Med* 2019;67:37–48. https://doi.org/10.1016/j.jflm.2019.08.001.
28. Twigg LE, Lowe T, Martin G. Sodium fluoroacetate residues and carcass degradation of free-ranging feral pigs poisoned with 1080. *Wildl Res* 2005;32:573–580. https://doi.org/10.1071/WR05026.
29. Chenais E, Depner K, Guberti V et al. Epidemiological considerations on African swine fever in Europe 2014–2018. *Porc Health Manag* 2019;5:6. https://doi.org/10.1186/s40813-018-0109-2.
30. National Agricultural Biosecurity Center Consortium Carcass Disposal Working Group. *Carcass disposal: a comprehensive review*. Kansas, USA, USDA Animal & Plant Health Inspection Service, 2004 Report No.: 02-1001-0355-CA. https://core.ac.uk/download/pdf/5164738.pdf.
31. Olesen AS, Belsham GJ, Bruun Rasmussen T et al. Potential routes for indirect transmission of African swine fever virus into domestic pig herds. *Transbound Emerg Dis* 2020;67:1472–1484. https://doi.org/10.1111/tbed.13538.

32. Probst C, Gethmann J, Amler S et al. The potential role of scavengers in spreading African swine fever among wild boar. *Sci Rep* 2019;9:11450. https://doi.org/10.1038/s41598-019-47623-5.
33. Ryan E, Mackay D, Donaldson A. Foot-and-mouth disease virus concentrations in products of animal origin. *Transbound Emerg Dis* 2008;55:89–98. https://doi.org/10.1111/j.1865-1682.2007.01004.x.
34. Teo CH, Hamzah H, Hing HL et al. Decomposition process and post mortem changes: review. *Sains Malays* 2014;43:1873–1882. https://doi.org/10.17576/jsm-2014-4312-08.
35. Parsons HR. *The postmortem interval: a systematic study of pig decomposition in west central Montana [master of arts]*. Montana, USA, The University of Montana, 2009. https://scholarworks.umt.edu/etd/1058/.
36. Lee Goff M. Early post-mortem changes and stages of decomposition in exposed cadavers. *Exp Appl Acarol* 2009;49:21–36. https://doi.org/10.1007/s10493-009-9284-9.
37. Heaton V, Moffatt C, Simmons T. Quantifying the temperature of maggot masses and its relationship to decomposition. *J Forensic Sci* 2014;59:676–682. https://doi.org/10.1111/1556-4029.12396.
38. Charabidze D, Bourel B, Gosset D. Larval-mass effect: characterisation of heat emission by necrophageous blowflies (Diptera: Calliphoridae) larval aggregates. *Forensic Sci Int* 2011;211:61–66. https://doi.org/10.1016/j.forsciint.2011.04.016.
39. World Organisation for Animal Health. *African swine fever*. Paris, France, World Organisation for Animal Health, 2022. https://www.woah.org/app/uploads/2021/03/a-african-swine-fever-v2-0.pdf.
40. Animal Health Australia. *Response strategy: African swine fever (version 5.1)*. Canberra, ACT, Australian Veterinary Emergency Plan (AUSVETPLAN), 2022.
41. Knight AI, Haines J, Zuber S. Thermal inactivation of animal virus pathogens. *Curr Top Virol* 2013;11:103–119.
42. World Organisation for Animal Health. *Foot and mouth disease*. Paris, France, World Organisation for Animal Health, 2021.
43. Farez S, Morley RS. Potential animal health hazards of pork and pork products. *Rev Sci Tech* 1997;16:65–78. https://doi.org/10.20506/rst.16.1.992.
44. Paton DJ, Sinclair M, Rodríguez R. Qualitative assessment of the commodity risk for spread of foot-and-mouth disease associated with international trade in deboned beef: risk for spread of foot-and-mouth disease through beef trade. *Transbound Emerg Dis* 2010;57:115–134. https://doi.org/10.1111/j.1865-1682.2010.01137.x.
45. McKercher PD, Yedloutschnig RJ, Callis JJ et al. Survival of viruses in "prosciutto di Parma" (Parma ham). *Food Res Int* 1987;20:267–272. https://doi.org/10.1016/S0315-5463(87)71198-5.
46. Research group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease (EuFMD). *Report of the session of the research group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease (EuFMD)*. Greifswald, Insel-Riems, Germany, Food and Agriculture Organization of the United Nations, 2005 Report No.: EUFMD/RG/05. https://openknowledge.fao.org/server/api/core/bitstreams/1a0bfed3-dc66-49a8-b1b3-cdf22ff22b35/content.
47. Hayrapetyan H, Groot MN, Zwietering M. *Literature search of heat and acid inactivation parameters of viruses relevant for food waste treatment for recycling to pig feed*. Wageningen, The Netherlands, Wageningen University, 2019 Report No.: 641933. https://eu-refresh.org/sites/default/files/REFRESH%20supplementary%20report%20D6.7%20Heat%20and%20acid%20inactivation%20parameters.pdf.
48. Mielke SR, Garabed R. Environmental persistence of foot-and-mouth disease virus applied to endemic regions. *Transbound Emerg Dis* 2020;67:543–554. https://doi.org/10.1111/tbed.13383.
49. Animal Health Australia. *Response strategy: foot-and-mouth disease (version 5.3)*. Canberra, ACT, Australian Veterinary Emergency Plan (AUSVETPLAN), 2024. https://animalhealthaustralia.com.au/ausvetplan/.
50. Mubayi A, Pandey A, Brasic C et al. Analytical estimation of data-motivated time-dependent disease transmission rate: an application to ebola and selected public health problems. *Trop Med Infect Dis* 2021;6:141. https://doi.org/10.3390/tropicalmed6030141.
51. Pech RP, Hone J. A model of the dynamics and control of an outbreak of foot and mouth disease in feral pigs in Australia. *J Appl Ecol* 1988;25:63–77. https://doi.org/10.2307/2403610.
52. Liaw A, Wiener M. Classification and regression by randomForest. *R News* 2002;2(3):18–22.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site: http://onlinelibrary.wiley.com/doi/10.1111/avj.13440/suppinfo.

Data S1. Supplementary Information.

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