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Predicted foot and mouth disease virus and African swine fever virus inactivation within carcases undergoing field decomposition in three Australian climate zones

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Australia's large populations of feral and extensively farmed livestock pose challenges to implementing response plans in the event of an Emergency Animal Disease outbreak. This study aimed to determine if a "Destroy and Let Lie" approach to carcase disposal (leaving carcases *in situ* to decompose naturally after field euthanasia) would reliably inactivate Foot and Mouth Disease virus (FMDV) and African Swine Fever virus (ASFV) under Australian conditions. Ninety-five animals (24 each of cattle, sheep, goats and 23 pigs) were used across six trials, conducted in winter and summer, in three locations in Eastern Australia. After euthanasia, temperature and pH were measured at six internal anatomical sites hourly for 24 h, then less frequently for a further 24 h. Data were compared with published FMDV and ASFV inactivation thresholds to assess the likely effectiveness of field decomposition in reducing viral infectivity. Tissue pH levels generally declined for the first 6–12 h postmortem. Based on a pH threshold of <6, FMDV would be reliably inactivated in the thoracic and abdominal cavities and deep and superficial muscle sites. In contrast, no porcine tissues at any location in any season would provide inactivation of ASFV, based on a pH threshold of <3.9. "Destroy and Let Lie" appears to be a suitable approach to reduce risk of FMDV transmission from carcases that cannot be disposed of using conventional means under Australian field conditions. This would not be the case for an ASF outbreak, where expected viral inactivation would be minimal.

Keywords African swine fever; carcase disposal; decomposition; emergency animal disease; foot and mouth disease; viral inactivation

Abbreviations 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; WOAH, World Organisation for Animal Health

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Australia is free from most significant Emergency Animal Diseases (EADs), as defined by the World Organisation for Animal Health (WOAH) and acts to protect itself from the introduction, establishment and impacts of EADs.[1–3] One of the best defenses against EADs is effective and efficient response planning.[1,4] Several EAD threats are specifically noted for Australia, but Foot and Mouth Disease (FMD) and African Swine Fever (ASF) rank highly due to their significant impacts on animal health and welfare, the economy and their rapidly evolving global epidemiology.[5–7]

Australia's primary goal in the event of an EAD outbreak is rapid disease eradication and return to a disease-free status with minimal detrimental impacts, particularly with respect to loss of trade in livestock products.[8,9] Response to many EAD incidents, as outlined in the Australian Veterinary Emergency Plan (AUSVETPLAN),[4] is the enaction of a "stamping-out" policy, which includes the destruction of infected livestock, direct contacts or suspected cases.[8,9] In the event of FMD and ASF outbreaks, this may extend to populations of feral and extensively farmed livestock.[10] In cases of ASF, feral pigs can become long-term reservoirs and may also challenge control efforts in an FMD outbreak, along with feral goats, camels and water buffalo.[11–13] Australia has large populations of feral animals, with estimates of 3.2 million pigs, 2 million goats, 1 million camels and 150,000 water buffalo.[14–17] Extensively farmed or semifarmed livestock may also be a source in maintaining or transmitting EADs.[10]

A significant proportion of both feral and extensively farmed livestock populations in Australia reside in vast and remote areas with difficult terrain. The relative inaccessibility of these areas presents challenges for implementation of response activities.[10] Aerial culling is the method typically applied for stamping out under such circumstances, but carcase disposal remains a challenge.[18] Carcase disposal is critical in the event of a major EAD incident, as carcases can represent a source of ongoing disease spread.[19–21] Preferred methods are outlined in the AUSVETPLAN "Disposal" operation manual[19] and include on-site burial, landfill burial, burning or incineration, rendering and composting.[22] These methods, however, are not always practical or possible.[19]

The "Destroy and Let Lie" (D&LL) method is postulated as an alternative option for carcase management within an EAD incident response and involves leaving carcases *in situ* after field euthanasia.[23] It relies on natural postmortem physiological changes, specifically pH and temperature changes during decomposition, to inactivate

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viruses such as FMD virus (FMDV) and ASF virus (ASFV). An understanding of how quickly pathogens are inactivated in carcases under a range of relevant environmental conditions is required to determine the practical efficacy of this strategy. To date, such investigations in the field are limited. Typically, studies that have directly measured inactivation of FMDV or ASFV postmortem have done so with samples of tissues rather than whole carcases and/or at temperatures applicable to meat storage (≤4°C) or under controlled laboratory conditions.[20,24–28] Field inactivation studies have been undertaken on continents other than Australia, mostly in the northern hemisphere. Thus, none of the findings from these studies can be directly applied to the D&LL method under Australian field conditions. One preliminary study conducted in Queensland, Australia, provided some evidence that FMDV inactivation may be achieved in muscle and/or body cavities of carcases under Northern Australian conditions.[23] However, the authors recommended that further research was required, as sampling was limited to two anatomic sites per carcase, included very low numbers of sheep, goats and cattle, at only one location each, and the more temperate parts of Australia were not represented.

Determining if D&LL is a viable option for carcase management and pathogen inactivation has many benefits. Beyond representing an option where no other disposal method is feasible, it has the potential to save millions of dollars in carcase management costs, better protect the environment and be more time efficient, which is critical in large-scale EAD management events. Ongoing research in this area is required to extend previous findings,[23] determining if the physiological conditions within decomposing carcases are sufficient to inactivate key pathogens and better defining parameters (e.g., climatic, viral characteristics, body location) that influence inactivation. The D&LL process also needs validation for practical application under Australian field conditions.

The aim of this study was to determine if natural livestock carcase decomposition processes would effectively and reliably inactivate FMDV and ASFV within a practical timeframe under Australian conditions, to validate potential use of D&LL as an option for carcase management during an EAD incident response. The primary objective was to record pH and temperature measurements from representative tissues of carcases from predominant livestock species over a 48-h period postmortem. The second objective was to compare empirical carcase pH and temperature data against published FMDV and ASFV inactivation thresholds to forecast likely inactivation outcomes under Australian field conditions. The third objective was to investigate, within anatomical sites, associations between pH and temperature parameters and key study variables: species, season and geographic location.

Materials and methods

Study locations and experimental design

The study was conducted at three locations, representing a range of Australian climatic conditions and substantive livestock production areas: South West Queensland – Charleville, Queensland; North Queensland – Charters Towers, Queensland; and Northern Victoria – Rutherglen, Victoria. Mean summer and winter maximum and minimum temperatures, and mean six-monthly rain and climate classifications are shown in Table 1.

Six trials were conducted, one at each location in both winter and summer, between June 2022 and February 2023. For each animal trial, four animal carcases for each of four livestock species, cattle (*Bos taurus*), goats (*Capra aegagrus hircus*), sheep (*Ovis aries*) and pigs (*Sus scrofa*) (i.e. 16 animals per trial) were to be used. Within each trial, sampling of carcases occurred as two consecutive "runs" each comprising two animals of each species. Animal ethics approval for this research project was provided by the Department of Primary Industries QLD Animal Ethics Committee (ref no. SA 2022/03/823) and Department of Jobs, Skills, Industry and Regions VIC Agricultural Research and Extension Animal Ethics Committee (AEC Code No. 2022–06). Biosafety approval was provided by the University of Queensland Biosafety Committee (Reference IBC/521B/VET/2022).

Experimental animals

Animals were selected to be as similar as possible to each other. However, the final animal choice was also guided by purchase opportunities from market sales or through donation by feral pest control programmes. Although gender, specific age, breed, fleece coverage, health status, and other parameters of animals were not controlled through selection of animals, they were recorded.

Animals were housed, fed, and watered following production and welfare-appropriate conditions for each species for a period of up to 4 days before destruction. All animals were humanely destroyed at first light by close-range head shots using an appropriate calibre projectile. Animals' levels of stress and forced exercise were minimised before death to enhance welfare considerations and reduce inter-animal variability in postmortem physiology. After confirmation of death by a registered veterinarian, animals were moved to the designated sampling location, which was out of sight of the remaining live animals. Animal carcases were placed in left lateral recumbency, approximately 1 m from each other (Figure 1). Access to carcases by large scavengers was prevented with predator-proof fencing. Half of the study population, that is, one animal of each species in each run, also received an additional postmortem gunshot wound to the dorsal abdominal cavity. This was to investigate the potential effects of internal organ disruption on decomposition parameters, which was associated with an ancillary study that will be reported separately.

Sampling of carcases

Carcases were incised at standardised anatomical sites for tissue pH and temperature measurement access (Figure 2). The abdominal cavity was incised immediately ventral to the paralumbar fossa, roughly midspaced across the abdominal zone. The thoracic cavity was incised between the fifth and sixth ribs, halfway between the ventral and dorsal surfaces. Stab incisions were made 10 cm deep into the semimembranosus and/or semitendinosus muscles for deep muscle sampling. Stab incisions were made either more superficially in this area (cattle) or in the neck musculature (other species) for superficial muscle sampling. Skull gunshot wounds were used to access the brain, and long bones (both the femur and humerus) were midsectioned to access bone marrow for sampling. Incisions were made large enough to accommodate sampling equipment, but small enough to maintain the anatomical and physiological integrity of the site.

Table 1. **Mean maximum and minimum summer and winter temperatures, mean six-monthly rain and climate classifications, for each study location**

Location	Mean summer temperatures (°C)		Mean winter temperatures (°C)		Mean rainfall (mm)		Climate classification	
	Max	Min	Max	Min	Oct-march	April-sept	Temperature/ humidity	Seasonal rainfall
South West Queensland – Charleville	34.7	21.3	20.6	5.3	334	152	Hot dry summer, cold winter	Summer dominant
North Queensland – charters towers	33.7	21.6	25.3	11.7	521	139	Hot humid summer	Summer
Northern Victoria – Rutherglen	30.5	13.1	13.3	2.4	264	323	Warm-mild/warm summer, cold winter	Winter

Data were sourced from the Australian Bureau of Meteorology.[29,47]

Figure 1. **The placement of animal carcases at the designated sampling locations.**

Figure 2. **The location of incisions for sampling each carcase anatomical site, indicated by red circles. Bone marrow was sampled via probe insertion within long bones (as per femoral incision on this image). Brain sampling was conducted via another penetrative wound to the forehead (not visible in this image).**

pH and temperature were measured using a WP-80 pH sensor probe (TPS, QLD, Australia). Baseline ($T = 0$) measurements were taken immediately once carcases were prepared. Cavity and muscle measurements were then taken hourly for the initial 24 h postmortem and then at 27, 30, 33, 36 and 48 h after the initial measurement. Brain and bone marrow measurements were taken at 3, 6, 12, 24 and 48-h timepoints. The pH sensor probe was placed at least 100 mm deep into each sampling incision and rinsed with distilled water and disinfected with 1% Virkon (Antec International, Sudbury, UK) in between carcases. Temperature data were only recorded once the probe reading had stabilised. The abdominal cavity was measured last and with pH and temperature probes being disinfected to minimise contamination of subsequent sites.

Weather data

Weather data for the nearest weather station to each study site were sourced from the Australian Government Bureau of Meteorology[29]: Charleville – Charleville Aero Station Number 044021; Charters Towers – Townsville-Fanning River (Defence) Station Number 033328; and Rutherglen – Rutherglen Research Station Number 082039.

Virus inactivation thresholds

FMDV inactivation thresholds: Summary information and literature used for defining FMDV inactivation thresholds were sourced from Williams,[30] Bachrach et al.[31] Animal Health Australia[8] and WOAH.[32] For the pH parameter, FMDV was considered inactivated if any pH measurement for that site was <6 or >9.[32] For the temperature parameter, data from Bachrach et al.[31] for time to reduce infectivity by 1 $\log_{10}$ (as indicated by Log TC ID_{50} per mL) at five temperature recordings from 4 to 61°C were used to estimate the time required at interim temperatures by fitting a regression model with temperature as the outcome variable and $\log_{10}$ time[2] as explanatory variables. It was assumed that the time taken for a 2 $\log_{10}$ reduction was double that of a 1 $\log_{10}$ reduction. The imputed times to reduce infectivity by 1 $\log_{10}$ to 4 $\log_{10}$ for a range of temperatures over a maximum 48-hour time period were estimated. Time for reduction in infectivity was assumed to be cumulative rather than continuous. Each carcase for which FMD inactivation was not expected due to a minimum pH < 6 was categorised as expected to

have a 0, 1, 2, 3 or 4 $\log_{10}$ reduction in infectivity based on the maximum result for any of the thresholds considered. Further details are provided in the Supporting Information.

ASFV inactivation thresholds. For pH readings, ASFV was considered inactivated in a site within a carcase if any pH measurement was <3.9 or >11.5.[9] ASFV was considered inactivated if the temperature was estimated to be >56°C for at least 70 min or >60°C for at least 20 min.[9] The half-life of 0.41 days at 37°C reported by Davies et al.[33] was used to estimate the expected reduction in titre (measured in half-lives) based on estimated time above 37°C.

Data management

Implausible values. pH values were considered implausible if they increased by >1 unit from one hourly time point to the next then reduced by >1 unit by the following hourly time point, or vice versa. This was not applied to all bone marrow and brain data and the later data for other anatomical sites (i.e. for measurements with >1-hour intervals).

Sample point. For statistical analyses, each data entry value was assigned to a sample point based on time from baseline sampling, grouped hourly for the first 24 h, then at 27, 30, 33, 36 and 48 h.

Time above a given temperature. For each time period (between two samplings), for each site within each carcase, the time within that period above a given temperature (increments of 1°C from 29 to 49°C) was estimated assuming a linear change in temperature between the two sample points. The estimated cumulative time above a given temperature for each site within each carcase was calculated by summing the time above each temperature for each time period.

pH variables. A binary variable was created for each anatomical site, for each carcase, indicating whether or not a pH of <6 was recorded for at least one sampling point. Time to pH <6 was defined for each site for each carcase as the earliest time from baseline sampling that a pH of <6 was recorded.

Data analysis

Descriptive analysis. A suite of trajectory plots was used to visualise pH and temperature changes over time for combinations of each anatomical sampling site, species, location and season. Temperature plots included air temperature recordings to compare carcase and ambient temperatures. As the time of first sampling differed between carcases within runs, air temperature time zero was set as midway between the earliest and latest times of first sampling.

Estimated FMDV inactivation. The relative frequencies of FMDV inactivation for each site, species, location and season combination were tabulated with associated 95% confidence intervals, adjusted for clustering by site, where applicable.

Generalised estimating equation models. These were used to investigate differences in patterns of abdominal cavity and thoracic cavity temperature change over time across species, location and season. Location × sample point (as a categorical variable), species × sample point and season × sample point were fitted as the explanatory variables, with carcase ID as the clustering variable. Model specification included an exchangeable correlation matrix and robust standard errors.

Time to pH <6. For situations where pH <6 was achieved at some stage for most carcase sites tested (i.e. abdominal and thoracic cavities, deep and superficial muscle), median times to pH <6 were estimated. Cox proportional hazard regression models were fitted to examine possible associations between time to pH <6 and species, location and season with use of additional abdominal bullet included as a covariate. All fixed effects were included in all models as it was decided *a priori* that effect estimates for each variable adjusted for potential confounding of the other variables were of interest. The assumption of proportional hazards was tested.

Likelihood of pH <6. For situations where pH <6 was achieved less commonly (i.e. bone marrow and brain), the crude percentage of carcases with minimum pH <6 was calculated. Logistic regression models were fitted to examine possible associations between pH <6 and species, location and season, with the use of additional abdominal bullet included as a covariate. All fixed effects were included in all models as it was decided *a priori* that effect estimates for each variable adjusted for the potential confounding of the other variables were of interest.

Data management and analyses were conducted using Stata 18.0 (StataCorp. Stata Statistical Software: Release 18. College Station, TX: StataCorp LLC. [2023]).

Results

Study population and conditions

Overall, 95 rather than 96 animals were included in the study. For the Charleville winter sampling, only three pigs were available, and all were sampled during one run. Maximum and minimum temperatures during each run were within 4°C of season means for nine runs. Charleville summer run 2 temperatures were lower than typical, the minimum temperature during Rutherglen winter run 1 was lower than typical, and the maximum temperature for Rutherglen winter run 2 was higher than typical. There was no rain during 10 of the runs, 8 mm during Charleville summer run 1 and 0.2 mm during Rutherglen winter run 1.

Descriptive results

pH trajectories. Nine pH values were removed from the dataset due to implausibility. The superficial muscle pH readings for one cow had several >1 unit fluctuations, but as no individual data point was clearly the outlier, no changes were made.

Individual trajectory plots of pH at a given site for a given carcase typically had a moderate amount of "noise" relative to the total range of pH, with oscillations between successive sample points indicating that measurement error was likely to be moderate for these data. This level of variability meant that the capacity to identify patterns from individual trajectories was limited. However, some trends could be discerned. Typically, abdominal cavity, thoracic cavity, deep and superficial muscle pH had already declined by the time of the first sampling, then continued to reduce for approximately 12 h before increasing slightly. This pattern was less apparent among

goats, with the early drop in pH being much less marked. For the first 6–8 h, deep muscle pH was usually lower than the other sites. The lower frequency of bone marrow and brain sampling meant that a pattern was not so apparent for these sites, but pH tended to remain higher at these sites compared with others.

Temperature trajectories. Individual trajectory plots of temperature at a given anatomical site for a given carcase were typically smooth relative to the total range of temperatures, with relatively few marked decreases or increases between successive sample points, indicating that measurement error was likely to be lower for these data compared with pH.

A suite of trajectory plots for each location-season-species combination was produced showing temperature at each site for each animal. A lagged relationship was seen in most of these plots between air temperature and abdominal and thoracic cavity and deep and superficial muscle temperatures. A typical example, cattle at Charters Towers during the summer sampling, is shown in Figure 3. Deep muscle temperatures typically did not increase as much in response to a rise in air temperature as abdominal cavity, thoracic cavity and superficial muscle temperatures. The lower frequency of bone marrow and brain sampling meant that the above pattern was not so apparent for these sites. Despite these typical patterns, there were some inconsistent observations. For example, the superficial muscle temperatures among cattle at Charters Towers during the winter sampling were unusually but consistently low.

Extrapolation of pH and temperature results to inactivation of FMDV and ASFV

Generalised estimating equation models. Predicted average abdominal cavity temperature patterns for each of the parameter's species, location and season averaged over the other two variables are shown in Figure 4. Note that the models used for these predictions assume the effect of species was constant across locations and seasons, the effect of location was constant across season and species, and the effect of season was constant across species and location. Average temperatures were similar at sampling point zero but were subsequently consistently higher in summer compared with winter and generally higher at Charters Towers compared with Charleville and Rutherglen. The diurnal variability in temperatures was lowest in sheep and highest in pigs. Similar patterns were seen in equivalent models of thoracic cavity temperature.

Expected FMDV inactivation. pH values <6 were frequently recorded, but values >9 were not. Expected inactivation of FMDV due to pH <6 was common for abdominal and thoracic cavity and deep and superficial muscle sites, but less so for bone marrow and brain sites (Table 2). Overall, it was less common in goats than in other species. There was a great deal of variability between carcases. The number of anatomical sites in which inactivation was expected based on a minimum pH <6 within a carcase varied from zero (1 goat carcase) to six (2 cattle, 7 pigs and 4 sheep carcases) out of 6. These demonstrate the variability in FMDV inactivation probability based on pH.

Temperature played no additional role beyond that of pH in expected reduction in FMDV infectivity during the winter sampling period. There was some expected reduction in infectivity due to temperature during the summer sampling period in tissues where inactivation was not expected due to pH. This was mostly in bone marrow or brain, with a reduction of 1–3 logs.

Expected time to FMDV inactivation based on pH <6. This approach was considered for sites where pH <6 was achieved at some stage for most carcases. No evidence of a violation of the proportional hazard assumption was found for any of the Cox proportional hazard models presented. Median times from first sampling to pH <6 and the results from Cox proportional hazard models are shown for abdominal cavity, thoracic cavity, deep and superficial muscle sites in Table 3. Within anatomical sites, there was some variability in time to pH < 6 by species, location and season, but patterns were not consistent across sites.

Expected likelihood of FMDV inactivation based on pH <6. This approach was considered for tissue sites where pH <6 was not achieved at some stage for a significant proportion of sample points,

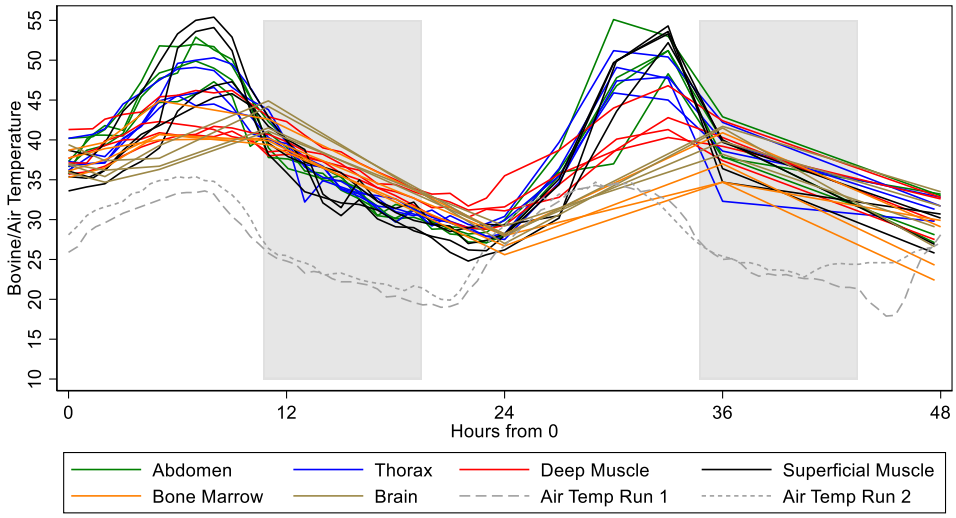

Figure 3. Exemplar trajectory plot of temperature for all anatomical sites from cattle at Charters Towers in the summer. Air temperature at Townsville-Fanning River (Defence) weather station for both runs is also shown. Shaded areas represent time from sunset to sunrise.

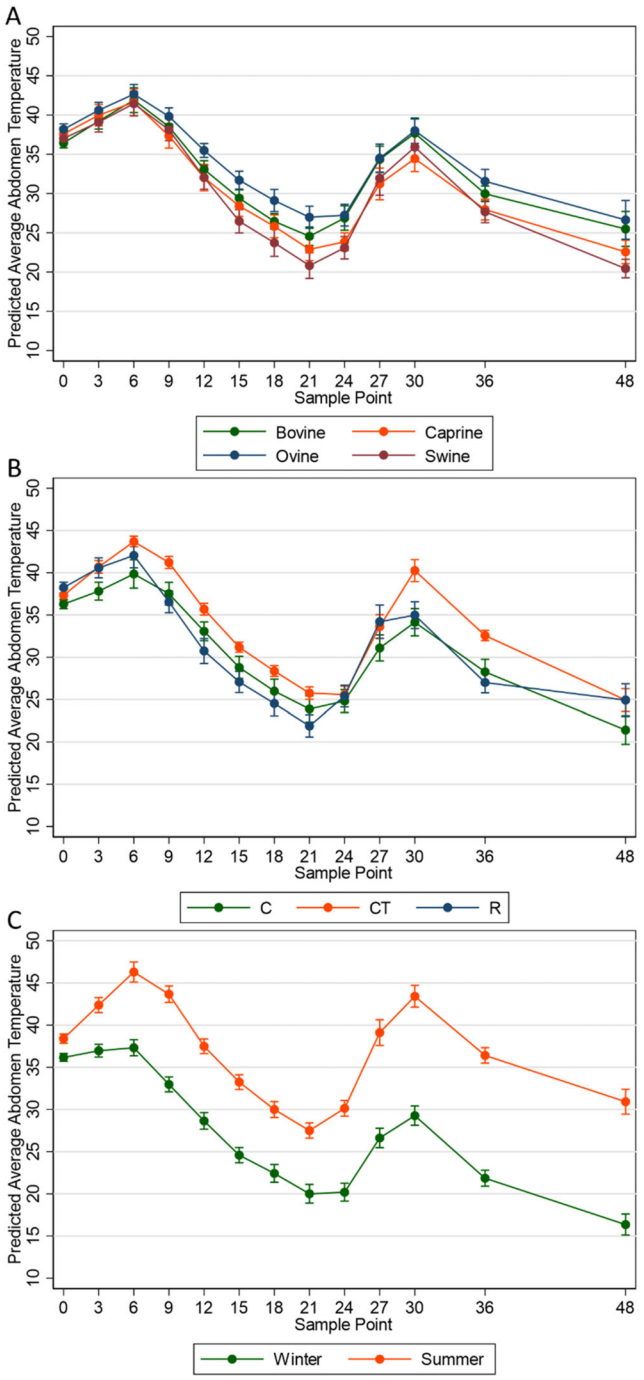

Figure 4. **Predicted average abdominal cavity temperature patterns from generalised estimating equation model (A) by species averaged over location and season, (B) by location averaged over species and season and (C) by season averaged over location and species. C—Charleville, CT—Charters Towers, R—Rutherglen.**

that is, bone marrow and brain. The crude percentage of carcases with minimum bone marrow and brain pH <6 and results from multivariable logistic regression models are shown in Table 4. Bone marrow was least likely to reach pH <6 in goats and at Charleville compared with the other sites. There was also some evidence that the likelihood of brain pH <6 varied by location and season.

Expected ASFV inactivation. No anatomical site within any pig carcase met the criteria for expected inactivation of ASFV during either the winter or summer sampling periods at any location, based on pH and temperature thresholds. In general, there was very little expected reduction in infectivity at any site during the winter sampling periods. However, in the summer, there was moderate expected reduction at most sites, most consistently in the abdominal cavity, thoracic cavity and superficial muscle sites, but less so in the bone marrow, and inconsistently in the brain.

Additional information

Additional information on the study population and example trajectory plots can be found in the Data S1.

Discussion

This study aimed to evaluate the potential efficacy of a D&LL approach to animal carcase management in the event of an EAD event within Australia. This was achieved by undertaking structured measurement of temperature and pH parameters within the tissues of decomposing livestock carcases and modelling survival of two paradigm EAD pathogens (FMDV, ASFV) based on published inactivation data from previous studies.

pH trajectories and analyses

pH levels generally declined from normal physiological levels for the first 6–12 h postmortem, before stabilising or rising again, as is consistent with other postmortem tissue pH data and studies.[34,35] For most anatomical sites for most carcases, declines in pH reported in this study were sufficient for expected FMDV inactivation, but this was never the case for ASFV. This finding is consistent with published measurements[23] of carcase central and peripheral pH across the same range of species.

Species differences. The decline in pH in the abdominal cavity and superficial muscle sites was markedly slower to reach <6 in goats compared with other species. Similarly, the likelihood of bone marrow reaching pH <6 was also much lower in goats. This finding was largely consistent with that from published measurements,[23] reporting that goats were less likely than other species to attain a central or peripheral pH ≤6.

These species differences could relate to body size, as goat and sheep carcases generally weighed less than the other species. It is a common consensus that smaller carcases progress to the end stage of decomposition more rapidly than larger carcases, which is likely to result in more rapid declines in pH.[36–38] Species variability could be more related to relative tissue predominance and nutritional and functional differences. There is also the potential confounding factor of variability in perimortem stress, as this would influence glycogen reserves and therefore decline in pH from glycolysis.[39]

Anatomical site differences. For the first 6–8 h, deep muscle pH was typically lower than the other sites, likely representing higher rates of conversion of glycogen to lactic acid in these heavily-muscled sites during rigor mortis.[40,41] The lower frequency of bone marrow and brain sampling meant that a pattern was not so apparent for these sites, but pH tended to remain higher at these sites

Table 2. Relative frequency of expected inactivation of foot and mouth disease virus by anatomical site based on pH <6 presented by species, season and location with associated 95% confidence intervals (adjusted for clustering by site for overall total)

Species/season/location	Abdomen %	Thorax %	Deep muscle %	Superficial muscle %	Bone marrow %	Brain %
Cattle						
Winter						
Charleville	75.0 (19.4–99.4)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	25.0 (0.6–80.6)	50.0 (6.8–93.2)
Charters towers	75.0 (19.4–99.4)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	25.0 (0.6–80.6)	50.0 (6.8–93.2)
Rutherglen	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	50.0 (6.8–93.2)	0.0 (0.0–60.2)
Overall	83.3 (27.4–98.5)	100.0 (73.5–100.0)	100.0 (73.5–100.0)	100.0 (73.5–100.0)	33.3 (9.1–71.5)	33.3 (1.9–92.6)
Summer						
Charleville	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	50.0 (6.8–93.2)	25.0 (0.6–80.6)
Charters towers	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	25.0 (0.6–80.6)	50.0 (6.8–93.2)
Rutherglen	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	75.0 (19.4–99.4)	75.0 (19.4–99.4)
Overall	100.0 (73.5–100.0)	100.0 (73.5–100.0)	100.0 (73.5–100.0)	100.0 (73.5–100.0)	50.0 (7.7–92.3)	50.0 (7.7–92.3)
Goats						
Winter						
Charleville	75.0 (19.4–99.4)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	0.0 (0.0–60.2)	0.0 (0.0–60.2)
Charters towers	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	75.0 (19.4–99.4)	25.0 (0.6–80.6)	25.0 (0.1–99.0)
Rutherglen	25.0 (0.6–80.6)	75.0 (19.4–99.4)	75.0 (19.4–99.4)	50.0 (6.8–93.2)	0.0 (0.0–60.2)	0.0 (0.0–60.2)
Overall	66.7 (2.7–99.3)	91.7 (9.1–99.9)	91.7 (9.1–99.9)	75.0 (9.9–98.8)	8.3 (0.1–90.9)	25.0 (0.1–99.0)
Summer						
Charleville	50.0 (6.8–93.2)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	75.0 (19.4–99.4)	0.0 (0.0–60.2)	50.0 (6.8–93.2)
Charters towers	75.0 (19.4–99.4)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	75.0 (19.4–99.4)	0.0 (0.0–60.2)	50.0 (6.8–93.2)
Rutherglen	75.0 (19.4–99.4)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	25.0 (0.6–80.6)	50.0 (6.8–93.2)	25.0 (0.6–80.6)
Overall	66.7 (28.5–90.9)	100.0 (73.5–100.0)	100.0 (73.5–100.0)	58.3 (6.8–96.4)	16.7 (0.1–97.2)	41.7 (14.0–75.7)
Pigs						
Winter						
Charleville	100.0 (29.2–100.0)	100.0 (29.2–100.0)	100.0 (29.2–100.0)	100.0 (29.2–100.0)	0.0 (0.0–70.8)	33.3 (0.8–90.6)
Charters towers	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	75.0 (19.4–99.4)	75.0 (19.4–99.4)
Rutherglen	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	50.0 (6.8–93.2)	0.0 (0.0–60.2)
Overall	100.0 (71.5–100.0)	100.0 (71.5–100.0)	100.0 (71.5–100.0)	100.0 (71.5–100.0)	45.5 (2.4–96.5)	45.5 (1.0–98.5)
Summer						
Charleville	75.0 (19.4–99.4)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	0.0 (0.0–60.2)	75.0 (19.4–99.4)
Charters towers	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	50.0 (6.8–93.2)
Rutherglen	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	75.0 (19.4–99.4)
Overall	91.7 (9.1–99.9)	100.0 (73.5–100.0)	100.0 (73.5–100.0)	100.0 (73.5–100.0)	66.7 (0.3–99.9)	66.7 (28.5–90.9)
Sheep						
Winter						
Charleville	75.0 (19.4–99.4)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	75.0 (19.4–99.4)	25.0 (0.6–80.6)
Charters towers	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	50.0 (6.8–93.2)	50.0 (6.8–93.2)
Rutherglen	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	25.0 (0.6–80.6)	0.0 (0.0–60.2)
Overall	91.7 (9.1–99.9)	100.0 (73.5–100.0)	100.0 (73.5–100.0)	100.0 (73.5–100.0)	50.0 (7.7–92.3)	25.0 (1.2–90.1)
Summer						
Charleville	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	50.0 (6.8–93.2)	50.0 (6.8–93.2)
Charters towers	50.0 (6.8–93.2)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	75.0 (19.4–99.4)	25.0 (0.6–80.6)
Rutherglen	75.0 (19.4–99.4)	100.0 (39.8–100.0)	100.0 (39.8–100.0)	75.0 (19.4–99.4)	75.0 (19.4–99.4)	25.0 (0.6–80.6)
Overall	75.0 (9.9–98.8)	100.0 (73.5–100.0)	100.0 (73.5–100.0)	91.7 (9.1–99.9)	66.7 (28.5–90.9)	33.3 (9.1–71.5)

Deeper shading indicates higher probability of inactivation (0%–24%, 25%–49%, 50%–74%, 75%–99%, 100%).

Table 3. Median times (in hours) from first sampling to abdominal cavity, thoracic cavity, deep and superficial muscle pH <6 and results from Cox proportional hazard models (hazard ratios and associated 95% confidence intervals [CI]) estimating the associations between species, location and season on time for anatomical site pH <6

Variable/category	No.	Median time (hrs) to pH <6 (95% CI)	Hazard ratio (95% CI)[a]	P value
Abdominal cavity				
Species				**<0.001**
Bovine	24	3.0 (1.0–4.6)	Ref	
Caprine	24	13.3 (5.3 – >48)	0.3 (0.1–0.6)	<0.001
Ovine	24	7.2 (2.8–23.3)	0.5 (0.3–0.9)	0.03
Swine	23	1.1 (0.1–3.1)	1.9 (1.0–3.5)	0.05
Location				**0.002**
Charleville	31	15.0 (5.4–29.7)	Ref	
Charters towers	32	1.0 (0.1–4.0)	3.5 (1.9–6.4)	<0.001
Rutherglen	32	3.0 (1.2–6.5)	2.5 (1.4–4.5)	0.002
Season				**0.763**
Winter	47	4.5 (3.1–7.2)	Ref	
Summer	48	4.0 (1.9–9.0)	0.9 (0.6–1.5)	0.763
Thoracic cavity				
Species				**<0.001**
Bovine	24	5.0 (2.0–7.8)	Ref	
Caprine	24	4.3 (1.0–6.8)	1.5 (0.8–2.7)	0.201
Ovine	24	4.3 (2.8–5.8)	2.0 (1.1–3.7)	0.021
Swine	23	1.3 (0.8–3.4)	3.9 (2.0–7.4)	<0.001
Location				**0.002**
Charleville	31	4.8 (3.6–8.1)	Ref	
Charters towers	32	2.3 (0.8–4.8)	2.8 (1.6–4.8)	<0.001
Rutherglen	32	3.4 (1.8–5.8)	1.7 (1.0–2.9)	0.056
Season				**0.006**
Winter	47	4.5 (3.0–6.7)	Ref	
Summer	48	3.0 (1.3–4.8)	1.9 (1.2–2.9)	0.005
Deep muscle				
Species				**0.101**
Bovine	24	1.4 (0.8–2.1)	Ref	
Caprine	24	0.8 (0.0–1.9)	1.0 (0.6–1.9)	0.899
Ovine	24	0.0 (0.0–1.3)[b]	1.4 (0.8–2.5)	0.249
Swine	23	0.0 (0.0–0.1)[b]	2.0 (1.1–3.7)	0.027
Location				**0.196**
Charleville	31	1.0 (0.0–1.4)	Ref	
Charters towers	32	0.0 (0.0–0.8)[b]	1.4 (0.8–2.4)	0.185
Rutherglen	32	1.0 (0.0–1.9)	0.9 (0.5–1.5)	0.637
Season				**0.653**
Winter	47	0.8 (0.0–1.0)	Ref	
Summer	48	0.0 (0.0–1.3)[b]	0.9 (0.6–1.4)	0.653
Superficial muscle				
Species				**<0.001**
Bovine	24	2.8 (1.3–3.0)	Ref	
Caprine	24	6.6 (3.8 – >48)	0.2 (0.1–0.4)	<0.001
Ovine	24	2.8 (1.8–5.7)	0.7 (0.4–1.2)	0.188
Swine	23	3.7 (2.3–5.4)	0.6 (0.3–1.0)	0.061

Table 3. Continued

Variable/category	No.	Median time (hrs) to pH <6 (95% CI)	Hazard ratio (95% CI)[a]	P value
Location				**0.031**
Charleville	31	3.7 (3.0–6.6)	Ref	
Charters towers	32	2.8 (1.8–3.8)	2.0 (1.1–3.4)	0.015
Rutherglen	32	3.3 (2.0–6.7)	1.0 (0.6–1.8)	0.886
Season				**0.261**
Winter	47	3.7 (3.0–4.1)	Ref	
Summer	48	3.0 (1.8–5.0)	1.3 (0.8–2.0)	0.262

[a] All models included species, location, season and use of additional abdominal gunshot as fixed effects.
[b] 0.0 represents baseline sampling.
Overall likelihood ratio P values for each variable are bolded; individual Wald P values for each category relative to the reference category are in normal font.

Table 4. Crude percentage of carcases with minimum bone marrow and brain pH <6 and results for logistic regression models (odds ratios and associated 95% confidence intervals [CI]) estimating the associations between species, location and season on minimum pH <6

Variable/category	No.	Crude min % pH <6	Odds ratio (95% CI)[a]	P value
Bone marrow				
Species				**0.008**
Bovine	24	41.7	Ref	
Caprine	24	12.5	0.2 (0.0–0.8)	0.024
Ovine	24	58.3	2.1 (0.6–7.3)	0.223
Swine	23	56.5	1.9 (0.5–6.3)	0.325
Location				**0.05**
Charleville	31	25.8	Ref	
Charters towers	32	46.9	3.0 (0.9–9.8)	0.062
Rutherglen	32	53.1	4.2 (1.3–13.6)	0.018
Season				**0.078**
Winter	47	34.0	Ref	
Summer	48	50.0	2.3 (0.9–6.0)	0.078
Brain				
Species				**0.224**
Bovine	24	41.7	Ref	
Caprine	24	33.3	0.7 (0.2–2.3)	0.53
Ovine	24	29.2	0.5 (0.2–1.9)	0.342
Swine	23	56.5	1.9 (0.6–6.5)	0.302
Location				**0.061**
Charleville	31	41.9	Ref	
Charters towers	32	53.1	1.6 (0.6–4.7)	0.354
Rutherglen	32	25.0	0.4 (0.1–1.3)	0.14
Season				**0.099**
Winter	47	31.9	Ref	
Summer	48	47.9	2.1 (0.9–5.2)	0.099

[a] All models included species, location, season and use of additional abdominal gunshot as fixed effects.
Overall likelihood ratio P values for each variable are bolded; individual Wald P values for each category relative to the reference category are in normal font.

compared with others. This is consistent with the finding[23] that in pig carcases, brain pH was less likely to reach ≤6 compared with central and peripheral sites. In the event of application of the D&LL approach, risks associated with reduced likelihood of virus inactivation at these sites are balanced by relative inaccessibility of these bone-protected tissues to predators and other influences that would disseminate infectious materials.

Location differences. Compared with other locations, a more rapid decline in pH was observed at Charters Towers in several anatomic sites. Reasons for this are not clear, but may be related to the physiology of the animals used at this location or other reasons unrelated to geography. However, this may be related to differences in climatic zones, such as the level of relative humidity which affects the rate of decomposition and, therefore, postmortem pH changes within tissues. One study[42] observed rapid decomposition of pig carcases in the tropical climate of Townsville, Northern Queensland compared with previous studies in the more southern and western areas of Australia. Further research should aim to differentiate such location effects based on climatic/geographical differences as opposed to physiological differences of animals selected at this location. It should be noted that there is confounding between these parameters, as the species and breeds of livestock do vary considerably based on geographic and climatic region.

Season differences. The lower rate of pH decline/lower probability of reaching pH <6 in winter compared with summer in several anatomic sites is likely related to postmortem tissue physiological changes associated with differential ambient temperatures. Seasonality, as well as other extrinsic factors such as relative humidity, rainfall and ambient temperature are also well-known influential factors of decomposition and have been extensively observed in existing literature.[43–45] In the event of application of the D&LL approach to a FMDV outbreak in winter, there is some evidence that lower levels of viral inactivation in both the brain and bone marrow would be projected, as compared with an outbreak in warmer weather. This would need to be accounted for when managing carcases under different seasonal conditions. These differences may not be critical, depending on the length of time carcases are left in the field to decompose.

Temperature patterns

This study provides strong evidence that ambient climatic conditions affect all tissue temperatures. This was identified from the apparent lagged relationship between air temperature and abdominal cavity, thoracic cavity, deep and superficial muscle temperatures, higher average abdominal cavity temperatures in summer compared with winter, and higher average abdominal cavity temperatures at Charters Towers compared with Charleville and Rutherglen. Temperature is expected to play a much lower role in FMDV inactivation compared with pH, but higher temperatures are expected to contribute somewhat to the reduction in infectivity of ASFV.

Anatomical site differences. Deep muscle temperatures typically did not increase as much in response to a rise in air temperature as abdominal cavity, thoracic cavity and superficial muscle temperatures. This is consistent with a carcase decomposition study[44] that found deeper tissues of human cadavers and pig carcases had decreased variability in temperatures compared with those more superficial, which they attributed to the limited exposure to ambient temperature.

Species differences. The lower diurnal variability in average abdominal cavity temperatures in sheep is likely to reflect the insulation provided by wool cover, whereas the higher variability in pigs may reflect the lower levels of insulation provided by sparse hair cover, particularly as the pigs from Charleville and Charters Towers were of smaller size due to breed and age differences associated with stock availability. Although animal subjects were chosen to be as homogenous as possible, within species differences occurred. This was particularly the case for the pigs, where feral animals were used in some locations, although domestic animals were used in other locations, based on availability and contextual relevance. Future studies should endeavour to examine feral and domestic pigs as separate entities with respect to decomposition parameters.

Study limitations

A key limitation of this study was the use of pH and temperature measurements to model virus inactivation rather than using infected carcases and directly measuring virus status over time. This approach was required for this field study as FMDV and ASFV are exotic to Australia and cannot be the subject of direct field trials. Existing studies measuring inactivation of these viruses assess the effect of temperature at a constant pH or pH at a constant temperature in isolated tissue, fluid or faecal samples, so findings cannot be directly applied to dynamic pH and temperatures of whole carcases in a field setting. It is likely that the thresholds applied in this study are conservative, particularly because reduced pH and increased temperature would be expected to have a synergistic effect, so virus inactivation may actually be expected earlier or more likely to occur than estimated. Nonetheless, data on other parameters would be needed to more accurately predict the risk of transmission of virus to other animals from carcases. Another limitation to this study was the relatively small sample size, given the large within-species between-animal variability during individual runs. However, this was limited by resources, time and ethical animal use considerations. Measurement error and influence of the measuring procedure on the true values in an undisturbed carcase should also be considered. It is possible that there will have been some inaccurate low pH measurements that have been interpreted as sufficient for virus inactivation where the true value would not have been sufficient.

Future research

A specific opportunity for future research would be to evaluate pH and temperature changes over longer-term decomposition periods, as the effects of these parameters on viral stability are time-dependent, so long-term exposure to even moderate levels of pH and temperature insult may further reduce the viral load in carcases. A study that allows for differentiation of the effect of temperature and pH, and any combinatorial or synergistic effect, would be valuable, though challenging to conduct.

Conclusion

The key finding from this study is that D&LL appears to be applicable in the event of an FMD outbreak, based primarily on pH characteristics. D&LL does not, however, appear to be a reliable approach to manage ASF transmission risk from carcases, be it based on pH or temperature parameters. This is not surprising, given the well-recognised hardiness of ASFV to physical and chemical inactivation, especially when it is contained in a protein matrix, particularly compared with FMDV.[30–32,46] However, this study presents specific viral

survival data that can be used to further model potential effects of decomposition parameters on viral survival and derive applied carcase management protocols.

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Conflicts of interest and sources of funding

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Data availability statement

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

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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.70002/suppinfo.

Data S1. Supporting Information.

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