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## SID 5 Research Project Final Report



01 June 2009

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## **Project identification**

1. Defra Project code A

Project title

2.

AW0820

Transcontinental road transport of breeder pigs - effects of hot climates

- 3. Contractor organisation(s)
  4. Total Defra project costs (agreed fixed price)
  4. Total Defra project costs
  - 5. Project: start date ..... 01 February 2006

end date .....

SID 5 (Rev. 3/06)

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

### **Executive Summary**

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

#### Scientific objectives

1. To determine the range of thermal conditions that breeder pigs encounter during transcontinental transport by road.

2. To characterise the physiological and potential welfare consequences of these conditions and journeys.

3. To define the acceptable ranges and limits for thermal conditions for breeder pigs during transcontinental road transport.

4. To provide the sound scientific basis for negotiation and development of future welfare legislation and codes of practice relating to the transportation of pigs.

The project was originally scheduled to commence in February 2006 for completion in January 2009 over a period of three years. The project was designed to consist of three distinct phases (corresponding to each year of the project).

- (1) The first year or phase would focus upon characterisation of the thermal micro-environments on commercial vehicles carrying breeder pigs on transcontinental export journeys in Europe. Long distance slaughter journeys in mainland southern Europe would also be examined in order to obtain further information pertaining to hot weather, long distance transport and the associated on-board thermal micro-environments. In addition the nature, design and performance of typical mechanical ventilation systems employed on higher standard vehicles as used for export or transcontinental transport of breeder pigs would be reviewed.
- (2) The second phase or year 2 would involve experimental journeys using a single vehicle and the same drivers on a number of shipments of 100 kg gilts (to simulate breeder pig transport) on a number of replicate journeys from the UK to southern Spain in summer. The objective was to characterise the on-board thermal micro-environments (and compare these to the data obtained in phase/year 1) and to measure and monitor the physiological and behavioural responses of the pigs during and after transport. By correlation of the animal measures with the thermal loads imposed in transit the severity of the various thermal conditions imposed during commercial type transportation (breeder export journeys in hot weather) could be

determined. This would constitute the development of a field based model of the responses of breeder pigs to heat loads in transit on long journeys. This in turn would contribute to the identification of acceptable range and limits for thermal loads during transport of breeder pigs.

(3) In the final phase (year 3) the intention was to utilise the data from years/phases 1 and 2 to design climate chamber based experimental studies in which thermal loads (typical of those encountered in commercial transport, and above that range, in controlled increments) could be imposed upon experimental pigs at the appropriate stocking densities in transport pens. This would allow extension to a wider range of controlled thermal loads and refinement of the field model developed in phase 2.

Despite very significant success in phases or years 1 and 2, in August and September of 2007 the UK was affected by outbreaks of Foot and Mouth Disease (FMD). This effectively terminated the planned experimental export journeys and only three actual journeys were completed. In discussions with Defra it was concluded that the effectiveness of the field modelling approach, even when based upon only three completed journeys, was such that this constituted an important route for progress in determining acceptable thermal envelopes for the transportation of breeder pigs.

Following extensive discussions the original project proposal was revised and the intended phase 3 was postponed. The new research plan involved a year 3 devoted to experimental journeys from the UK to Spain as previously intended for completion in year/phase 2. As a great deal was learned from the field experiments (experimental journeys) during phase/year 2, many changes and improvements to the original experimental protocols from the preliminary studies. It was therefore proposed that the new phase 3 would constitute the definitive study of the effects upon breeder pigs of transcontinental transport in hot weather. Thus the field model for definitions of acceptable ranges and limits for thermal loads in transit for breeder pigs would be based on the 7 journeys undertaken in the summer of 2008.

The report includes three sections each devoted to a separate phase or year of the project:-

| Section 1 | Thermal micro-environments on commercial vehicles carrying breeder pigs on transcontinental export journeys in Europe   |
|-----------|---|
| Section 2 | Preliminary studies on three experimental journeys from the UK to Spain (terminated by the FMD outbreaks)   |
| Section 3 | Seven experimental export journeys from the UK to Spain and development of the field model and definition of acceptable thermal enveloped for long distance export journeys for breeder pigs. |

PHASE 1 – YEAR 1

During the first phase of the project the thermal conditions within vehicles on journeys across Europe were quantified on six commercial shipments of breeder pigs. On each of these shipments data were recorded (air temperatures and relative humidities) at various locations throughout the vehicle (at pig level) and for ambient conditions.

On typical journeys from France to Greece during July, temperatures within the vehicle in excess of 30°C were reached. There was a clear diurnal rhythm in both temperature and water vapour density. Night-time minima and day-time maxima increased as the vehicle travelled in to the warmer conditions encountered in southern Europe. During the ferry journey from Italy to Greece, high thermal loads were generated within the vehicle, probably as a consequence of both elevated external temperatures and reduced ventilation of the "bio-load" whilst on the ship.

During the warmer weather encountered upon the July journey, 71% of the temperature readings were 20-30°C, indicating markedly increased thermal loads upon the pigs in transit. On this journey "on-board" temperatures in the range 30-35°C occurred for 4% of the time. Therefore it was concluded that on this particular mid-summer journey, pigs would have been exposed to temperatures at or above the current recommended maxima ( $30^{\circ}C \pm 5^{\circ}C$ ) for significant periods.

A commercial shipment of pigs was also monitored within Spain on a 12-hour journey. This was deemed to be important as, aside from establishing commercial collaborators in Spain that were essential for later phases; the journey was representative of the proposed final phase of an export shipment travelling to southern Spain. On this journey ambient temperatures in excess of 38°C were recorded. For more than 9 hours of the 12-hour journey external or ambient temperature was greater than 30°C. It was apparent that at the end of the journey that the pigs exhibited a mild degree of hyperthermia, but that the measured

surface temperatures indicated a more severe hyperthermia may have occurred in transit. Despite this finding all the pigs appeared in good health upon arrival and there were no mortalities, apparent severe stress or pathologies in the pigs when inspected in the delivery or recovery pens prior to slaughter. This journey was considered representative for the proposed final phase of breeder pig export journeys and provided a good basis for the second phase of the project.

Measurements were also made to characterise the mechanical (fan) ventilation performance of livestock vehicles. By comparison with wind-driven ventilation, the fans are equivalent to that provided by a crosswind of 1.2 m/s, with the fan covers on, and 1.5 m/s with the covers off. Wind-driven ventilation is likely to be reasonably uniform throughout the pens as all are equally exposed. However, the distribution of ventilating air may not be uniform with the fans operating with the covers off, as there is significant air movement between pens. This is not the case with the fan covers on, where there is little air movement between pens. When parked in the open, cross wind flow is likely to dominate and fan ventilation is only likely to make a significant contribution in near still conditions.

#### PHASE 2 – YEAR 2

Three, long-distance, hot weather, export journeys with pigs were successfully completed in this phase. A complementary control non-transport study was also undertaken. The on-board (vehicle) and external climatic thermal environments were fully monitored and characterised for all journeys and these could be classified by the thermal loads imposed on the pigs in transit. The thermoregulatory and other physiological responses of sentinel (implanted) and accompanying pigs were monitored and measured on all journeys and inter-journey differences were described. The post-transport behaviours observed over a 4-hour period differed significantly between journeys, reflecting differences in the severity of the demands and stressors imposed during the journeys. This was the first study to fully integrate environmental, physiological and behavioural measures in the long distance (export) transport of breeding pigs for the purpose of developing a predictive model that will allow definition of acceptable ranges and limits for thermal loads on such journeys. The study forms the sound basis for developing such a model relating animal welfare, behaviour and physiological stress to transport conditions and practices under commercial export conditions.

#### PHASE 3 YEAR 3

In the third and final phase or year of the project, seven experimental journeys carrying 100 kg gilts from the UK (Ellenthorpe, Yorkshire) to southern Spain (a slaughterhouse in Humilladero, Andalucía) were completed successfully during the summer of 2008. The journeys were designated by sequential numbers/identifiers from J21-27. A number of physiological and behavioural indices were employed to assess the responses of the pigs to transport and to the range of thermal micro-environments to which they could be exposed to in a hot season. The effects of the journeys and the conditions upon the animals were assessed by:-

- o Body temperature responses
- Drinking behaviour/hydration state
- Resting behaviour/fatigue

These indices were integrated and correlated with the physical or thermal classification of each journey (based on temperature and enthalpy). There was a high degree of consistency between the different methods of classification between the separate approaches. Integration of the physiological and behavioural data indicated that most adaptive responses occur in the three journeys (23, 24 and 25) that took place in July and August (high summer). It is suggested that some degree of transport stress was associated with these journeys and the thermal conditions within the vehicle. It is proposed that the thermal conditions on the vehicle during the last phase of the journey (last 22 hours) had a major influence upon post-transportation behaviours and physiology of the pigs. Journeys 21, 22 and 27 (conditions) did not impose excessive transport stress upon the pigs. The experimental journeys covered a typical range of thermal conditions and transport micro-environments for Southern Europe, and were consistent with those observed and reported in phase 1 and 2 of the project for both the commercial and experimental journeys carrying breeder pigs and gilts.

The thermal conditions encountered included journeys that might be classified as "mild", "warm" and "hot". A number of physiological and behavioural measures and analyses were correlated with the vehicle thermal micro-environment, and the potential stress imposed upon the pigs was assessed. On none of the journeys was severe thermal stress identified. Indeed all the pigs appeared in good health and condition upon arrival. The deep body temperature did not exhibit any major excursions outside the normal ranges recorded in the home pen, apart from those correlated with feeding and arousal during lairage. Journeys that were scored highly on elevated temperatures and enthalpy were associated with

some changes in both physiological and behavioural indices of stress. Thus journeys 23 and 24 (and perhaps 25) were associated with some degree of thermal stress (not severe). It appeared that the journeys undertaken in July (J23 and 24) were associated with the biggest risk of thermal stress and the expression of physiological and behavioural responses associated with the elevated heat loads. Journeys undertaken in June and August (J22, 25 and 26) imposed some degree of heat stress and caution should be exercised when transporting animals under these conditions, because these journeys had periods when conditions were in the upper ranges for temperature currently prescribed in the Regulation (EC 1/2005). The journeys undertaken in early June and in October (J21 and J27) when ambient temperatures were less than in high summer imposed little, if any, thermal stress and consequently constituted little risk to the well being or welfare animals. Whilst journeys undertaken in conditions close to or at the limits of temperature prescribed in the current Regulation (EC 1/2005) were associated with some physiological adaptive responses these did not constitute a major threat to the welfare of the animals

The results suggest that if transportation is undertaken in a manner consistent with current legislation, on appropriate vehicles and with high standards of personnel and practice, there is little threat to the welfare of the pigs even in relatively hot conditions (typical of Southern Europe in summer). It is suggested that the prescribed temperature limits are revisited and reviewed in order that future legislation may take full cognizance of the animal's physiological and behavioural responses and sound scientifically based definitions of acceptable thermal envelopes for transportation.

This work has provided the sound scientific methodology and basis of a model, which, with appropriate further research and model development can inform future policy and legislation or regulations and can provide a platform for negotiation by the UK government and Defra at planned future discussions on European guidelines and regulations on animal transportation.

Current legislation (EC 1/2005) contains an upper limit of  $30^{\circ}C \pm 5^{\circ}C$  for the transportation of all livestock on journeys of over 8 hours duration. This study has demonstrated that on higher standard vehicles with adequate ventilation and a high standard of animal care, temperatures of around  $30^{\circ}C$  need not necessarily impose undue heat stress upon breeder pigs in transit. It is therefore suggested that to provide a sound scientific basis for any revision of the Regulation, future research studies should focus upon the effects upon animals of temperatures in the range  $28^{\circ}$  to  $36^{\circ}C$  (with a range of appropriate relative humidities) for appropriate ranges of periods of exposure. This will provide indications of what temperature/humidity combinations would be acceptable for each of the exposure periods. Only through such an approach will it be possible to ensure the welfare of animals in transit in hot weather through the provision of precise limits for imposed thermal loads and durations of exposure. These issues should be addressed through both modelling studies and further concomitant field trials and it is recommended that this be a major research priority.

## **Project Report to Defra**

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

## Defra project code AW0820

### Transcontinental road transport of breeder pigs - effects of hot climates

### FINAL REPORT – JUNE 2009

#### Scientific objectives

1. To determine the range of thermal conditions that breeder pigs encounter during transcontinental transport by road.

2. To characterise the physiological and potential welfare consequences of these conditions and journeys.

3. To define the acceptable ranges and limits for thermal conditions for breeder pigs during transcontinental road transport.

4. To provide the sound scientific basis for negotiation and development of future welfare legislation and codes of practice relating to the transportation of pigs.

The project was originally scheduled to commence in February 2006 for completion in January 2009 over a period of three years. The project was designed to consist of three distinct phases (corresponding to each year of the project) as follows:

**Phase 1.** The first phase or year 1 would focus upon characterisation of the thermal micro-environments on commercial vehicles carrying breeder pigs on transcontinental export journeys in Europe. Long distance slaughter journeys in mainland southern Europe would also be examined in order to obtain further information pertaining to hot weather, long distance transport and the associated on-board thermal micro-environments. In addition the nature, design and performance of typical mechanical ventilation systems employed on higher standard vehicles as used for export or transcontinental transport of breeder pigs would be reviewed.

**Phase 2.** The second phase or year 2 would involve experimental journeys using a single vehicle and the same drivers on a number of shipments of 100 kg gilts (to simulate breeder pig transport) on a number of replicate journeys from the UK to southern Spain in summer. The objective was to characterise the on-board thermal micro-environments (and compare these to the data obtained in phase/year 1) and to measure and monitor the physiological and behavioural responses of the pigs during and after transport. By correlation of the animal measures with the thermal loads imposed in transit the severity of the various thermal conditions imposed during commercial type transportation (breeder export journeys in hot weather) could be determined. This would constitute the development of a field-based model of the responses of breeder pigs to heat loads in transit on long journeys. This in turn would contribute to the identification of acceptable range and limits for thermal loads during transport of breeder pigs.

<u>Phase 3.</u> In the final phase or year 3 the intention was to utilise the data from phases 1 and 2 to design climate chamber-based experimental studies in which thermal loads (typical of those encountered in commercial transport and above that range in controlled increments) could be imposed upon experimental pigs at the appropriate stocking densities in transport pens. This would allow extension to a wider range of controlled thermal loads and refinement of the field model developed in phase 2.

Despite very significant success in phases 1 and 2 in August and September of 2007 the UK was affected by outbreaks of Foot and Mouth Disease (FMD). This terminated the planned experimental export journeys and only three actual journeys were completed. In discussions with Defra it was concluded that the effectiveness of the field modelling approach, even when based upon only three completed journeys, was such that this constituted an important route for progress in determining acceptable thermal envelopes for the transportation of breeder pigs.

Following extensive discussions, the original project proposal was revised and the intended phase 3 was postponed. The new research plan involved a year 3 devoted to experimental journeys from the UK to Spain as previously intended for completion in year 2 – phase 2. A great deal was learned from the field experimental protocols from the preliminary studies were implemented. It was therefore proposed that the new phase 3 would constitute the definitive study of the effects upon breeder pigs of transcontinental transport in hot weather. Thus the field model for definitions of acceptable ranges and limits for thermal loads in transit for breeder pigs would be based on the seven journeys undertaken in the summer of 2008.

This report focuses on the final year of the research programme. Previous reports are included as Appendices 1 (phase 1) and 2 (phase2).

All figures and tables for this report have been included in Appendices 3 and 4 respectively.

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### PHASE 3 – YEAR 3

#### Experimental journeys from UK to Spain

Development and application of a field model to assess the effects of hot weather transcontinental transport on "breeder pigs".

- (i) The background, objectives and logistics of year 3.
- (ii) Weather conditions at the departure location and the destination.
- (iii) Thermal conditions in transit (ambient and "on-board").
- (iv) Physiological and behavioural responses of the pigs.
- (v) Summary and conclusions.

## 1. The background, objectives and logistics of Year 3

The research objective was to try to ensure the scheduling of seven experimental journeys during summer 2008 were made over a range of conditions that were representative of "typical summer conditions" in Europe.

Each shipment of pigs comprised a total of 80 pigs (approximately 100kg liveweight) transported from the UK to southern Spain. Within each shipment were 20 sentinel pigs that had received prior treatments and 60 "ballast pigs" to complete the load.

All the pigs used in the research were sourced from the same farm in Ellenthorpe, North Yorkshire. All the pigs used in the study were organised into groups of 10 at an early age and remained in the same groups throughout the experiments.

A number of weeks prior to each shipment a batch of 20 pigs (two groups of 10) were transported from the source farm to the Large Animal Unit (LAU) of Roslin Institute, Edinburgh where they underwent surgery to implant data loggers to record deep body temperature.

#### NOTE: Animals surgically implanted with deep body temperature monitoring devices.

In this study up to 8 "sentinel pigs" per journey were surgically implanted with data logging or telemetry devices for the continuous recording of deep body temperature over a period covering the pre-transport phase and the entire journey up until slaughter. These procedures are regulated by the Home Office Animals (Scientific Procedures) Act 1986. The surgical procedures were extensively appraised and reviewed by the Animal Experimental Committee of SAC and the Animal Experimental Ethics Committees and Experimental Review Processes of both Roslin Institute and the University of Edinburgh. They were appraised also by ethical review in the Universidad Politécnica De Madrid. All procedures and protocols were approved by these bodies prior to submission of the project licence to the Home Office. All experimental and surgical procedures were approved by the Home Office and appropriate licences granted prior to the commencement of the study and were monitored by the Home Office throughout entire project. All procedures were performed at the appropriate Home Office designated sites. All animals were monitored closely after surgical implantation and full recovery from the procedure was confirmed prior to any further procedure. A minimum period of at least 14 days was allowed between surgical implantation and undertaking the experimental journeys.

Within each sub-group of 10, four pigs were implanted with data loggers so that for each shipment there were eight implanted pigs on the vehicle. The pigs were allowed to recover for 3 weeks prior to the shipment date. Throughout this report the experimental journeys have been described as commencing in Ellenthorpe in Yorkshire and terminating at the slaughterhouse at Humilladero in Andalucía, southern Spain. Ellenthorpe is close to the original source farm of all the pigs used in the studies and was the site of loading of all the ballast pigs, including the animals employed in the behavioural studies at the final destination. These animals were loaded on to the vehicle for the transport to Spain and later the animals for the physiological studies were also loaded. The 20 experimental pigs, including the sentinel animals that had been implanted with data loggers, were transported from Roslin in Scotland to Ellenthorpe to arrive and be cross loaded immediately prior to departure for the ferry and the onward journey. It is important to note that in compliance with WATO 2006 and EC 1/2005, the export journey officially commenced with the loading of the first pig in Roslin and NOT upon departure from Ellenthorpe. However, the "research journey" was considered to start at Ellenthorpe after the vehicle was fully loaded.

Shipments were made to Spain between June and October. Full details are given in Table 1.

On a shipment loading date, the sentinel pigs from Edinburgh were transported back to <u>near</u> the original source farm using a "shuttle truck". After it had been confirmed that the sentinel pigs had been loaded and had left Edinburgh, the main vehicle was loaded at Ellenthorpe with the remaining 60 pigs. Stocking density on the main vehicle was kept around 180 kg/m<sup>2</sup> which is typical of commercial practice when transporting breeder pigs on long journeys. The transport vehicles were all of the higher standard specification required for long journeys and had mechanical ventilation systems and on-board water drinkers.

The pigs were loaded, as groups of 10, onto the vehicle. There were nine pens distributed over three decks on the vehicle. The top deck front pen was left empty for spare bedding and feed that was needed during the journey.

The middle and rear pens on the middle deck were left empty pending the arrival of the sentinel pigs from Edinburgh. Once the main vehicle (Figure 1) was loaded with the 60 pigs it moved off the farm and parked away from the farm to await the sentinel pigs. The sentinel pigs were trans-shipped onto the main vehicle when they arrived (Figure 2).

Once the main vehicle was fully loaded, it left Ellenthorpe and drove overnight to Poole to await loading onto the morning ferry for the crossing to Cherbourg. On arrival at Cherbourg the vehicle drove on to the control post near Fougeres where the pigs were unloaded for the mandatory 24-hour rest period.

During the 24-hour rest period the pigs were fed regularly and had constant access to water. On completion of the 24-hour rest period the pigs were reloaded as the same groups into the same pens on the vehicle as previously.

The vehicle then drove down through France and Spain until arrival at the destination of Humilladero, north of Malaga. The total journey distance was around 3000 km.

On arrival, the two groups of sentinel pigs were unloaded first to enable physiological measurements to be made (see Section 4), with the remaining pigs then unloaded still in their original groups.

A typical journey profile is shown in Table2.

## 2. Weather conditions at the location of departure and at the destination

Records were kept of the ambient temperature in Edinburgh, Fougeres and Humilladero throughout the research period with measurements made every 30 minutes from late April to mid-October. These data loggers were located out of direct sunlight and away from any extraneous heat sources.

Figure 3 shows the daily mean temperatures in Edinburgh, Fougeres and Humilladero. The data are also presented in Figure 4 as the daily maximum temperatures at each location.

Over the period of measurement, the average daily mean temperature in Edinburgh was  $13.9 \pm 2.6$  °C, in Fougeres  $16.1 \pm 2.9$  °C and in Humilladero  $23.0 \pm 3.7$  °C. The corresponding maximum daily mean temperatures were  $18.7 \pm 3.4$  °C,  $21.9 \pm 4.0$  °C and  $28.0 \pm 5.5$  °C respectively. The actual maximum temperatures observed in the three locations were 26.8 °C (Edinburgh), 32.8 °C (Fougeres) and 37.0 °C (Humilladero). These maxima occurred at the end of August, late July and early August respectively. Comparison of these data with records obtained over the summer period during 2007, indicate that the mean and maximum daily temperatures in 2008 were consistent with the normal ranges encountered in these geographical locations.

It can be seen from Figures 3 and 4 that the dates of the shipments covered a range of ambient conditions that were typical of those occurring during a normal summer period. Thus, shipments 21 and 27 (June and October) took place when the differences in mean and maximum temperatures were least between the three geographical sites, whereas journeys 22-26 (July-September) took place when the temperatures in Spain were elevated and the differences between those values and the other two locations were much greater.

## 3. Thermal conditions in transit (ambient and "on-board")

Prior to loading the vehicle, data loggers (Onset computers, Hobo H8 loggers) were mounted on the partition gates between the pens on the middle deck. The data loggers were protected from direct contact and damage by the pigs by housing them in a perforated steel framework (Figure 5). The data loggers were pre-programmed to record, at regular (2 minute) intervals, the temperature and relative humidity of the air within the transport pens on the vehicle.

The measurements of air temperature and relative humidity were continuous throughout the whole transport period from Ellenthorpe to Humilladero. When the pigs were unloaded at Fougeres, though the data loggers remained on the vehicle, the conditions experienced by the pigs were very similar to those on the empty truck because of the open nature of the housing pens at the control post. In addition, there was good agreement between the conditions on the vehicle and those recorded by the ambient loggers.

The values of temperature and relative humidity were used to convert the values of relative humidity to vapour density to give an absolute measure of the moisture content of the air. The data were also used to derive the enthalpy content of the air, which is a measure of the total heat content of the air and gives a single measure for each data set of temperature and relative humidity.

In this report the values for the environmental/thermal variables are presented as the mean ± one standard deviation of the mean as well as the range indicated by the maximum and minimum values. This has been applied to the whole journey and to each journey phase or component. Implicit in this approach is that the data are normally distributed and therefore the mean value of a parameter for a journey phase or component, or for the whole journey, accurately reflects the thermal conditions experienced by the animals. Other measures of the average temperature might be employed, such as the median and the mode values. This approach has been adopted in Tables 3 and 4, where the results for all seven journeys are presented. In Table 3 all values for the whole of each journey for vehicle temperature, water vapour density and enthalpy are presented allowing a comparison to be made between the mean, median and mode values. In Table 4 the same approach has been adopted to analyse the thermal conditions on the vehicle during the final phase or the last 22 hours of each shipment. It is immediately apparent that the mean and median values are very close for all journeys (and indeed journey phases), and on this basis the thermal conditions in transit have been characterised by the mean values and ranges for the remainder of the report.

## 3.1 Journey phases and thermal parameters

Each of the seven experimental shipments was considered in six phases:

- (i) the overall journey from Ellenthorpe to Humilladero,
- (ii) from leaving Ellenthorpe to arriving at Poole,
- (iii) from arriving at Poole to leaving Cherbourg,
- (iv) from leaving Cherbourg to arriving at Fougeres,
- (v) the 24 hour rest period at Fougeres, and
- (vi) from leaving Fougeres to arriving at Humilladero.

The temporal variations of temperature and vapour density and temperature and enthalpy for the whole transport period are presented in Figures 6 to 19. For each journey or shipment two graphs are presented. The first graph of each pair contains plots of temperature and water vapour density for the entire journey period, and the second graph contains plots of temperature and enthalpy for the corresponding journey.

A distinct pattern with some obvious characteristics is exhibited generally for all the journeys. The temperature is relatively low at the commencement of the overnight component of the journey when the vehicle travels from the farm to the ferry. The temperature may increase during the period after sunrise and before boarding the ship. During the day-time ferry crossings, temperature in the vehicle tends to fall. This may be a consequence of the vehicle being stationed on an outside deck with good air flow, or ventilation through the animal compartments and a lower ambient temperature at sea.

As the ship approaches the harbour of arrival in France, the on-board temperature tends to increase. On arrival at the control post in France, the animals are unloaded and, of course, as the major heat and moisture source has been removed, the temperature and water vapour density within the vehicle decrease to equilibrate with ambient conditions. When the animals are reloaded at the end of the lairage period the on-board temperature then increases and continues to rise or exhibit a plateau stage for much of the journey through France (with the possible exception of shipment 26 where some lower temperatures were observed during this stage). There is some indication of an effect upon temperature during travel through the high altitude regions at the French-Spanish border.

Perhaps the most striking feature of the plots of temperature and enthalpy is the marked increase in on-board temperatures and enthalpies seen during the last 6-8 hours of the journey in Spain. This phenomenon is attributable to the combined effects of sunrise and the north to south nature of the journey at this stage, as the vehicle and its load pass into a warmer geographical region. On all journeys the temperature increase in this period was very obvious and it is clear that if there is a risk of heat stress, it is likely to be greatest during this phase of the shipments. Even on the cooler journeys this was apparent. On journeys 23-26 the temperature peaked in the last phase at a value of 30°C or greater. This maximum was reached after a marked and relatively constant rise over the final 6-8 hours. On journeys 21 and 22, the temperatures during the corresponding phase or period reached values between 25-30°C, and for journey 27, the peak was between 20-25°C. It may be concluded that the greatest risk of heat stress occurs during the last few hours of the journey and certainly within the final phase of the journey (Fougeres to Humilladero). It is proposed that this component of the journey could be analysed in more detail and related to animal responses as described in section 4 of this report.

For each phase of the journey, the data, (as presented in Figures 6-19), for temperature (°C), vapour density  $(g/m^3)$  and enthalpy (kJ/kg) were analysed for mean  $\pm$  SD, maximum and minimum values. The resultant data are shown in Tables 5 to 10.

The mean temperature within the vehicle over the whole transport period ranged from  $14.8 \pm 5.3^{\circ}$ C (Shipment 27) to  $22.2 \pm 3.6^{\circ}$ C (Shipment 23). The maximum and minimum temperatures recorded were  $34.2^{\circ}$ C (Shipment 24) and  $4.6^{\circ}$ C (Shipment 27) respectively.

The corresponding mean values of enthalpy ranged from  $30.1 \pm 7.7$  kJ/kg (Shipment 27) to  $47.1 \pm 4.9$  kJ/kg (Shipment 23). The maximum and minimum enthalpies recorded were 75.8 kJ/kg (Shipment 25) and 16.5 kJ/kg (Shipment 27) respectively.

As suggested above, the higher temperatures and thermal loads all occur in the final phase of the journey and this is clearly indicated in Figures 6-19. Therefore the data were further analysed to consider the final 22 hours of each shipment, which corresponded approximately to the period between leaving the control post at Fougeres and arriving at Humilladero. The temporal variations of temperature, vapour density and enthalpy for this 22-hour period are presented in Figures 20-26. As proposed previously, this interval might be considered the most thermally challenging as this is the period when the pigs experience the warmest ambient conditions.

Again, the plots of temperature, water vapour density and enthalpy for the last stage of the journey exhibit patterns that are generally similar across all seven shipments. Whilst both the mean and peak temperatures may differ in magnitude in the different journeys, the increases always occur in the last 6-8 hours. Enthalpy tends to mirror the temperature patterns (but see shipment 24) and this is primarily a consequence of fairly constant water vapour density values during this period. Water vapour density tended to be lowest in shipments 21 and 27 and elevated in the other journeys with highest values occurring during journeys 23 and 24. It is apparent that the higher temperatures on these journeys accompanied by elevated water vapour densities could potentially impose a much greater heat stress upon the pigs.

The analysed thermal data for this last stage of the journeys are presented in Figure 27 and summarised in Table 11. It is clear from the graphical presentation of the data in Figure 27 that the highest temperatures and enthalpies occur in the journeys that took place in July and August and that the cooler journeys were undertaken in June and October. The mean temperature was highest on Shipment 23 (23.7  $\pm$  2.8°C) and lowest on Shipment 27 (18.8  $\pm$  2.5°C). The maximum and minimum temperatures were 34.1°C (Shipment 24) and 10.8°C (Shipment 26) respectively.

## **3.2 Ranking of shipments**

Another possible approach in terms of ranking the journeys in relation to the thermal loads imposed in transit is to consider the mean temperature and temperature ranges encountered during the entire journey, i.e. from departure from Ellenthorpe until arrival in Humilladero. There are a number of reasons why this may not accurately reflect the effects of thermal conditions upon the transported animals' physiological and behavioural responses, but these will be considered in section 4. The total journey temperature and enthalpy values are presented in Table 5. On this basis the journeys (21-27) may be ranked in terms of mean and maximum temperature. Thus, based on mean temperature, the highest thermal load overall occurred in shipment 23 (late July) when mean temperature was  $22.2 \pm 3.6$ °C, and when maximum temperature is considered shipment 24 (early August) appeared to be the hottest journey (34.2°C). Ranking the shipments in order based on these criteria (highest to lowest thermal load) indicates that for mean temperature the journey order was 23, 24, 22, 25, 21, 26 and 27. For maximum temperatures recorded the order was 24, 25, 26, 23, 22, 21 and 27. These rankings will be discussed further below.

It was considered that the highest risk of thermal stress might occur during the final phase of the journey form Fougeres to Humilladero as the vehicle passed through southern France and in to Spain, and then south across the central plain and in to Andalucía. In this phase, the highest temperatures were encountered and high temperatures were sustained for the longest periods. This component of the journey took place after a 24-hour rest period in the control post, during which animals may have recovered, at least in part, from the first travel period in the UK. No extreme elevated temperature conditions were encountered during the lairage at the control post. For these reasons it was decided to analyse the thermal data for the last 22 hours of the journey from Fougeres to Humilladero.

To correlate the degree of thermal challenge imposed by each of these shipments during the final 22 hours, the ranking of the shipments according to the mean and maxima for both temperature and enthalpy was used. The former parameter was selected, as it is now a legal requirement that temperature within transport vehicles is recorded on long journeys, while the latter represents an integrated measure of the temperature and moisture content of the air. The resultant figures are shown in Tables 12 (mean values) and 13 (maximum values).

This analysis suggested that Shipments 23 and 24 potentially imposed the greatest thermal challenge on the pigs during the final 22 hours of the journey. However such an analysis is based on single values and makes no allowance for the duration of exposure to any given thermal load. To address this shortcoming the data were reanalysed and weighted according to an arbitrary scale, as shown in Tables 14 and 15. Each data point for the last

22 hours (660 readings) of temperature and enthalpy was ascribed a weighting score between 1 and 7. For each shipment the number of readings within each weighting band was totalled.

For example, in Shipment 21 the temperature data were:

| <ul> <li>307 readings with</li> <li>249 readings with</li> <li>104 readings with</li> <li>0 readings with</li> <li>0 readings with</li> </ul> | a weighting of<br>a weighting of<br>a weighting of<br>a weighting of<br>a weighting of<br>a weighting of<br>a weighting of | 1<br>2<br>3<br>4<br>5<br>6<br>7 |
|---|--|---------------------------------|
|---|--|---------------------------------|

A further index was calculated derived from the total sum of the weightings, so for the above date, the single index would equal:

(0x1) + (307x2) + (249x3) + (104x4) + (0x5) + (0x6) + (0x7) = 1777

The resultant weighted indices for temperature and enthalpy for all the shipments are presented in Table 16.

Though this approach may give a better integrated index of the total thermal load imposed on the pigs, it makes no allowance for the duration of individual periods of exposure to any "temperature range", as all values are totalled within a weighting band. In practice, this means that two periods of 10 consecutive readings would be scored the same a one period of 20 consecutive readings. This is not a true reflection of the physiological load imposed on the pigs and further investigation is needed to develop the concept of a "thermal dose". This is important in assessing the degree of thermal load and also in determining the duration of recovery necessary after any given exposure. It must be stressed that a prolonged episode of exposure to an elevated thermal load is likely to cause greater heat stress than a series of short exposures to the same heat load, separated by periods of exposure to reduced heat loads, during which some recovery may be possible. Recovery from heat stress during periods in lairage at control posts, at an abattoir or destination farm are an important aspect of the welfare of animals during transport, especially on long journeys, and this should be addressed further. The magnitude of the heat stress and the duration of exposure ("thermal dose") will undoubtedly influence the time required for recovery, as well as affecting the degree of physiological stress and the extent of behavioural responses exhibited by the animals during and at the end of the journey.

However, from physical thermal considerations alone, the current data suggest that the most challenging journeys seem to be Shipments 22, 23 and 24 and the least challenging Shipments 21, 26 and 27. This conclusion is based upon the relative rankings and derived scores for both temperature and enthalpy during the last 22 hours of each journey (see Tables 11 and 16).

The approaches described above have all facilitated the characterisation of the export journeys for pigs in terms of the thermal loads (temperatures, water vapour densities and enthalpies) and the ranking of the journeys in terms of the risk for inducing thermal stress in transported pigs. In order to better define the stress imposed, and therefore the risk of reduced welfare in the animals, it is necessary to examine the physiological and behavioural responses of the animals during and after transportation. By this method it is possible to correlate animal measures with the thermal micro-environment, and to compare journey categorisation based on environmental variables with an assessment of thermal conditions based on responses of pigs. Further, if extreme responses or marked changes in response pattern are identified relating to higher thermal loads, then it may be possible to employ the journey categorisation to identify limits for temperature (or thermal loads in transit) and the acceptable ranges for these variables in which animals may be safely transported. The animal measures and responses are presented and discussed in section 4 of this report.

## 3.3 Summary of thermal conditions during transport

The characterisation and analysis of the physical or thermal environment on the vehicles on all seven journeys are summarised below:-

- (1) The ambient temperatures and humidities have been measured throughout seven pig export journeys from the UK to Spain. The corresponding water vapour densities and enthalpies have been derived for each journey
- (2) The journeys were undertaken in a range of ambient thermal conditions in the summer and early autumn (June to October) and a parallel range of on-board thermal micro-environments were fully characterised

- (3) Inspection of the data indicated that the biggest risk of heat stress appear to be posed by the final phase of the journeys (regardless of the month of the shipment), or the last 22 hours of travel to the slaughterhouse
- (4) It was possible to use the thermal micro-environment data to categorise the journeys in terms of the predicted threat to the pigs in terms of heat stress
- (5) The categories identified were compared with subsequent analyses of physiological and behavioural data to establish the effects of each heat load range (each journey), and to identify those conditions which impose mild, moderate and severe thermal stress on the pigs in transit
- (6) If a high degree of stress is identified in response to any of the thermal regimes that occurred, then this can be used to establish an upper limit for thermal conditions for pigs in transit
- (7) This approach constitutes the basis of a predictive model that allows definition of the acceptable ranges and limits for thermal loads during the long distance transportation of breeder pigs

Based upon the physical or thermal environment alone and using the whole journey, and the last phase of the journey, it has been possible to produce an overall ranking in relation to potential risk of thermal stress. Thus, in descending order of predicted thermal challenge the ranking would be:-

#### Journey 23>24>22 >25 >26 >21 >27

Furthermore, it is possible to ascribe categories based on general meteorological principles and approaches to this ranking. As the data cover a relatively small range in terms of means and maximum temperatures, it is possible to apply categories in different ways, however the most useful and appropriate are those presented below (by journey number). Whilst journeys 23 and 24 may be regarded as hot, journey 22 may be regarded as either hot or warm. The remaining journeys clearly fall into the arbitrary grouping of warm or cool.

| НОТ  | 23 | 23 | НОТ  |
|------|----|----|------|
| НОТ  | 24 | 24 | НОТ  |
| WARM | 22 | 22 | НОТ  |
| WARM | 25 | 25 | WARM |
| WARM | 26 | 26 | WARM |
| COOL | 21 | 21 | COOL |
| COOL | 27 | 27 | COOL |

It should be stressed that on all seven journeys temperatures were reached on the vehicle which would be considered warm or hot in relation to normal summer temperatures in the UK. This is particularly true for the last phase of 22 hours. Based upon daily average and maximum temperatures at the departure location for the journeys, the "warm" journeys had mean and maximum temperatures that were around 5-7°C greater, and on journeys designated as "hot" the mean and maximum temperatures were 7-15°C higher.

However, the arbitrary designations or categories are useful in the identification of where the greatest risks to welfare might lie and the effects of the different thermal loads. Furthermore the appropriateness of the categories can be determined by inspection and integration of the animal measures (physiological and behavioural responses) as described in section 4.

## 4. Physiological and behavioural responses of the pigs

## 4.1 Physiological methods

The logistics of the experimental export journeys from the UK to Spain have been described in detail above. The seven shipments were undertaken between June and October 2008 and a range of thermal conditions were encountered. The journeys have been categorised according to the physical conditions in terms of temperature, water vapour density and enthalpy and according to a scoring and ranking system, as well as on the basis of the absolute values of the thermal variables. It is important to emphasise that the ambient conditions and thermal micro-environments on the vehicle reported in this section (phase 3 – year 3 of the study) are not only typical of southern Europe in the summer months, but are consistent with the values for temperature and humidity reported in phases 1 and 2 of the study for both commercial and experimental journeys. The physiological and behavioural responses to journeys and the associated categorised thermal micro-environments have been assessed and measured and form the basis of a predictive field model. The primary experimental objective was to determine how hot weather transport and the range of imposed thermal loads influenced the thermoregulatory status of the animals and to assess the degree of stress imposed by the conditions on the pigs. This was done by examining a number of physiological variables during and after the journeys, and behaviours after the journey

The measured variables included "spot measures" of rectal temperature, surface temperature and body temperature, by means of an implanted RFID chip, before and after transport as described below. In addition, continuous measures of deep body temperature were made using surgically implanted data loggers. These methods were improved and refined from those used in phase 2/year 2 and were employed in a larger number of pigs than in the previous studies. These measures of thermoregulatory status were complemented in later journeys by the use of thermal imaging to assess local and average body temperatures of the pigs. All measures were made both prior to the journey during a control period (for every pig) and at the end of the journey immediately upon arrival at the slaughterhouse in Spain. All the behavioural observations and recordings were made over a 3-4 hour period in the lairage in Spain.

In preparation for each of the seven journeys, eight pigs were surgically implanted with data-loggers (see section 2 and Defra projects AW0815, AW0922 and AW0925) for the continuous monitoring and recording of deep body temperature. All surgically implanted pigs and a further 12 pigs had radio-frequency identification chips (RFID) implanted in a sub-cutaneous site (3 cm deep) on the back of the neck by injection which allowed instant identification of individuals (20 pigs per shipment in total) using a chip reader and which also measured deep body temperature. This approach was fully validated in a previous project (Defra project AW0507). Pig surface temperatures were also measured by non-contact infra-red thermometry (Defra project AW0925) and rectal temperatures for purposes of comparison were also measured.

Saliva samples were taken for estimation of cortisol content and blood samples were taken at slaughter for estimation of plasma creatine kinase (CK) activity as an indicator of transport stress-induced muscle damage. Preliminary studies revealed that the measurements of CK activity in plasmas prepared from these blood samples were unreliable. Activities of the intracellular enzyme were greatly elevated in all samples and highly variable and did not reflect changes induced by the transport alone. This was a consequence of the necessary logistics of the experiment and slaughterhouse procedures. The pigs arrived during the afternoon on a non-slaughter day and could not be killed until the following day; this constraint was imposed by the slaughterhouse management. It was also necessary to hold the experimental pigs for behavioural studies for a period of at least 4 hours post-journey. It was deemed unacceptable to take blood samples from the pigs in lairage at the end of the journey, as this would involve restraint and stress. The only point at which blood samples cold be obtained was at slaughter. The 18-hour period between arrival and slaughter, and the handling (use of electric prods), stunning process (gas stunning using  $CO_2$ ) and muscle damage in the neck during the bleed out procedures and contamination at slaughter.

All physiological measures were applied both before and after the journeys (measurements made immediately upon arrival at the destination in Spain), whilst the implanted body temperature devices allowed continuous measurement of this variable in the pre- and post-journey periods (up until point of slaughter) and throughout the entire journey. In phase 3 of the project deep body temperature recordings were made over a pre-journey period that exactly corresponded to the subsequent period of travel for comparative purposes. Thus, if in the journey week animals travelled from Wednesday night until Saturday afternoon, then the body temperature patterns (daily rhythms, maxima, minima, mean values, etc.) were all estimated in time matched control periods for the previous week with the pigs in the home pens. Of course, during this control period the pigs were fed and watered and the

pens cleaned in accordance with normal farm procedures and best practice. In addition to the physiological and behavioural measurements, observational skin lesion scores were obtained before and after transportation or before and after the appropriate control period.

The methods used for the phase 3 field trials may thus be summarised as below.

## **4.2 Procedures and Measurements**

- Implant 8 sentinel pigs per journey with body temperature data loggers
- 4 sentinel pigs placed in each of two pens on the vehicle same pens on every journey
- 20 pigs per journey implanted with RFID transponders (measuring deep body temperature) designated "Chip Temperature" or CT
- 20 pigs sampled pre- and post-journey for Surface Temperature(ST), Rectal Temperature (RT)
- Saliva and blood samples taken from 20 experimental pigs
- 20 pigs (two pens of 10 not the experimental pigs) were used for the behavioural observation and recordings (video)

## 4.3 Measurement schedules

- Control measurements on experimental pigs were made pre-journey at origin Dryden (Roslin)
- Measurements during the journey (in transit) were confined to deep body temperature using the implanted data loggers
- Post-transport measurements were made on the tailgate of the vehicle and in the lairage in the slaughterhouse (Humilladero)
- ST and CT were measured on the tail-lift of the vehicle immediately upon arrival at the slaughterhouse
- All the behavioural observations and recordings (scans, focal, continuous) were made in holding pens in the slaughterhouse lairage, as were measurements of RT
- Saliva samples were obtained and skin lesion scores (single observer) were undertaken in the lairage pens
- Blood samples were obtained and implant recovery undertaken the following day at the point of slaughter

Actual measured variables:

- Rectal temperature
- Surface temperature
- Saliva
- Blood (plasma)
- PCV (packed cell volume)
- Deep Body Temperature data logger (logger implant)
- Deep Body Temperature RFID chip injection (+ identification)
- Behavioural analysis (recording)
- Lesion scores

As described in section 3.1, it is possible to express a variable in terms of a mean value and an estimate of error (e.g.± one standard deviation or SD) and a range, including a minimum and maximum value. Often it is useful to examine the effects of a treatment upon a variable by determining other estimates of the average value, such as the median and mode. Such estimates may be useful in any subsequent ranking or modelling applications depending upon the normality of distribution of the variable. This approach can be applied to body temperature in much the same manner as it was applied to the environmental thermal conditions. This procedure may be summarised

Body temperatures and environmental thermal data were expressed and analysed thus:-

- Mean ± one standard deviation
- Minimum values
- Maximum values
- Median values
- Mode values
- The values were assigned a score by magnitude and a product derived from the number of values in each range and that score
- Analysis was performed over the whole journey period and over of last stage (22 hours)
- Models of responses were derived from both absolute values and ranges/scores

## 4.4 Results

The results will be presented in the sequence of the spot or point physiological measures, the continuous monitoring of deep body temperature in transit, the effects of transport upon body weight, lesion scores and packed cell volumes (PCVs), and finally the behavioural responses of the pigs to the conditions and transport on the seven experimental journeys.

The integration of the physiological and behavioural analyses then allows categorisation of the journeys in terms of apparent thermal or transport stress imposed. Finally, correlation of the thermal environment categories with the biological or animal measures to facilitate determination of those thermal loads that impose unacceptable stress (if any) can be carried out. The data can then underpin recommendations for acceptable ranges and limits of temperature or thermal loads for the long distance transportation of breeder pigs in hot weather.

All figures and tables relating to the physiological and behavioural measures are presented in Appendices 3 and 4. The journeys are identified by sequential numbers as elsewhere in this report. As this was the second group of experimental journeys, the first, second, third journeys, etc. were designated as J21, J22, J23, etc. However, some of the commercial data analysis packages were limited to a single digit identifier for the journeys so the use of J1, J2, J3, etc. in figures and tables denotes the same phase 3 journeys.

## 4.4.1 Physiological measures – animal responses to transport and thermal micro-environment

#### Point or spot measures – comparison of pre- and post-journey values

The values of mean surface or skin temperatures of the pigs pre- and post-journey, along with the resulting mean changes, are presented in Table 17 and presented graphically in Figure 28 for the seven experimental journeys. It is apparent that the pre-journey surface temperatures varied little. The lowest value ( $29.1^{\circ}$ C) was on the October journey when ambient temperature was lowest in the UK, and the highest ( $33.6^{\circ}$ C) was recorded prior to Journey 24 (in July). There was much greater variability in the post-journey surface temperatures. Again the lowest was observed in Journey 27 ( $39.1^{\circ}$ C) and highest in Journey 24 ( $40.1^{\circ}$ C). The greatest change in surface temperature occurred in Journey 24 ( $+6.6^{\circ}$ C) and the lowest in Journey 27 ( $+2.3^{\circ}$ C). There were highly significant effects (p<0.001) of transportation upon skin or surface temperature, and highly significant differences (p<0.001) attributable to journey (i.e. the changes induced in this variable by transportation differed between journeys). There appeared to be a possible anomalous large increase in surface temperature in Journey 21. Other than this, the skin temperature changes appeared to be greatest in the July journeys (23 and 24) and lowest in the final journey in October. The patterns of these responses are presented in Figure 28 and it is clear that the July journeys (J23/J3 and J24/J4) are associated with elevated skin temperature at the point of arrival.

The deep body temperatures (DBT) pre-and post-journey determined from the implanted/injected RFID chips are presented in Table 18 and Figure 29. These measures were made, along with the skin temperatures measurements, whilst the pigs were on the tail lift of the vehicle, immediately upon arrival at the slaughterhouse in Spain. It is apparent that no major changes in DBT occurred on any journey based on this method of measurement. On the first journey (J21/J1) there was no change in DBT and apparent decreases occurred on Journey J22 and J27. On the shipments in July and August there were apparent but very small increases in DBT. There were highly significant differences (p<0.001) in post-journey DBT between journeys with the maximum change and highest value being associated with J23 and J24 respectively.

Rectal temperatures (RT) were also measured before and after transport, but the measurements were made in lairage a few minutes after arrival at the slaughterhouse and not on the tail lift of the vehicle. The data are presented in Table 19 and Figure 30. The pre-journey values were within the normal range expected. There was some evidence of slightly low, but not abnormal, values prior to transport for Journeys 26 and 27. Mean rectal temperature was unaffected in Journey 21 and only very small changes were observed in Journeys 21 and 27. The highest value of post-journey mean rectal temperature was observed on Journey 25. The largest increases occurred in Journeys 24 and 26. As can be seen from Table 19 and Figure 30, the variations in pre- and post-journey values for RT follow similar patterns. Only on Journeys 24 and 26 were relatively low pre-journeys values accompanied by high post-journey values resulting in the largest observed changes. The differences between post-journey values of RT differed significantly (p<0.001). There was a highly significant difference between the changes in RT on the different journeys (p<0.001).

A summary and comparison of the mean changes in surface/skin temperature (ST), RFID chip temperature (DBT) and rectal temperature (RT) observed in each of the seven journeys is presented in Figure 31.

<u>Surface or skin temperature:</u> J1, J4 and J6 show significantly greater change in temperature than J2, J3, J5 and J7 (P<0.001), but J1, J4 and J6 are not significantly different from each other. J2, J5 and J7 show significantly less change in surface temperature (P<0.001) than the other journeys but do not differ from each other. J3 shows significantly more change in temperature than J2, J5 and J7 (P<0.001), but less change than J1, J4 and J6.

<u>RFID Chip temperature or DBT</u>: J1, J2 and J5 show no significantly different change in chip temperature from each other. J3, J4 and J6 show a significant increase in chip temperature post-journey compared with J1, J2 and J5 (P<0.001). J7 shows a significant drop in chip temperature post-journey compared to all other journeys, except J2 (P<0001).

<u>Rectal temperatures</u>: J1, J2, J3, J5 and J7 show no significant change in rectal temperature post-transport to each other. J4 and J6 show a significant increase in rectal temperature post-transport compared to all other journeys (P<0.001).

The effects of transport upon pig body weights are presented in Table 20 and Figures 32 and 33. The lowest prejourney mean body weight was seen in Journey 23 (88.4 kg) and the highest in Journey 27 (107 kg). The changes in body weight caused by transportation were highly variable (see Figure 33). Losses of body weight occurred in Journeys 22, 24, 25 and 27. The latter weight loss was the greatest in the study. There were apparent body weight increases on Journeys 21, 23 and 26. The increases in body weight though variable were significantly different form the losses (p<0.001). The largest decrease observed on Journey 27 was significantly different (p<0.001) from all other journey-induced changes.

The data relating to the skin lesion scores (count) pre- and post-journey are presented in Table 21 and Figures 34 and 35. There were no data for Journey 23 as only limited pen space was available, and the experimental pigs were placed in a single pen (n = 20 not 2 times n = 10). The aggression and fighting that ensued compromised accurate lesion scoring. There were significant changes in lesion scores associated with transportation (p=0.002), the most striking effect being the apparent increases that were observed in Journeys 24 and 27 (see Figure 35).

The PCVs (%) are presented in Table 22 and Figure 36. PCV could not be measured until at least 18 hours after arrival at the slaughterhouse and thus all the pigs had been watered. PCV is a useful index of dehydration but on this occasion as the pigs had rehydrated could not be used for this purpose. PCV values were compared more as an indicator of general health and well-being of the pigs and all the values fell within the normal expected range with no significant differences between journeys.

#### Summary of point measures

From the point measures of the range of variables made upon arrival at the slaughterhouse in Spain (and for comparison with pre-journey values) some important features may be extracted. The measurements of skin or surface temperature may be considered to be an indicator of the transfer of heat from the core to the periphery. Thus when an animal experiences an elevated heat load, decreases in peripheral insulation or transfer of heat to the periphery by the circulation raises the skin temperature. Skin temperature may increase in the absence of any change in core temperature reflecting the efficiency of the enhanced heat loss mechanisms. Thus the higher skin temperatures recorded upon arrival at the slaughterhouse may be associated with an increased demand for sensible heat loss resulting from the thermal micro-environment on the vehicle. It should be noted that skin temperature will also be raised if the animal is exposed to radiant gain (e.g. solar gain or secondary gain from hot vehicle structures), and thus the measurements of ST upon arrival were made as quickly as possible to avoid this effect.

The data indicated that the July journeys (J23/J3 and J24/J4) were associated with elevated skin temperature at the point of arrival, and may have involved the imposition of a degree of heat stress or an increased requirement for sensible heat loss.

Similarly the journeys in which the maximum change and highest value of RFID Chip temperature were observed were J23 and J24 respectively. The RFID chips are injected 3 cm deep in the neck and have previously been shown (when employed in this manner) to yield useful estimates of DBT under heat stress conditions. As the sensors are not truly in the body core the values may be slight underestimates, but do reflect a degree of heat stress and heat transfer from core to periphery.

The rectal temperature measurements were not made until the pigs were transferred to the holding pens in the lairage in the slaughterhouse and some degree of heating or cooling might be imposed by the intervening period. Only on Journeys 24 and 26 were relatively low pre-journeys values of RT accompanied by high post-journey values resulting in the largest observed changes.

Overall it can be concluded, on the basis of the point or spot body temperature measurements, that significant heat loads, resulting in changes in values of ST, DBT and RT, were imposed on the pigs during the July journeys when compared to the other shipments. There may be lesser degrees of heat stress on the other journeys but this must not be dismissed or discounted. The minimal changes tended to be associated with Journeys 21 and 27 when thermal loads (from measurements of environmental conditions) were lowest. Thus a preliminary ranking of the journeys based on body temperature measurements can be undertaken. It is suggested, therefore, that the highest risk of heat stress occurred on Journeys 23 and 24, there was an intermediate risk on Journeys 22, 25 and 26, with the lowest risk being associated with Journeys 21 and 27.

There were no major or important conclusions concerning the effects of transportation or the on-board thermal micro-environments that could be drawn from the measures of body weight, lesion scores or packed cell volumes.

#### Continuous measurement and monitoring of Deep Body Temperature (DBT) by surgically implanted data loggers

On each of the seven journeys, eight sentinel pigs, previously implanted with data loggers in the peritoneum, were employed to record DBT throughout a control period (with the pigs in the home pen) and throughout the entire journey period, up to the point of death approximately 18 hours after arrival at the slaughterhouse. Four sentinel pigs were placed in each of the rear two pens on the middle deck of the vehicle on every journey. This approach allowed comparisons of body temperature values and the daily rhythms in DBT in matched periods (approximately 72 hours) under control conditions and in transit for the UK to Spain. The body temperatures were sampled at 2 minute intervals throughout. The resulting data were expressed as mean values (± one standard deviation) for the entire journey period and for each phase of the journey as previously discussed in the analysis of the thermal environments on the vehicle. As for the thermal environment data, the median and mode values were calculated to test the suitability of using mean values and to examine the necessity to express the averages by a measure other than the mean. Data for the entire journey period and the final phase of the journey (which was examined in detail) are presented in Table 23a and 23b.

It was clear that the median and mean values were always very similar and therefore (as for the thermal environment data) mean values were subsequently employed throughout. Again, consistent with analyses applied to the thermal micro-environments, the mean body temperature values and the changes during transport were "scored" by means of weighting factors (proportional to the deviation from the mean range determined for the entire study) to assess the severity of any thermal stress imposed in transit. Then the journeys were ranked on this basis and in relation to absolute values and changes in DBT. Comparisons of the mean and ranges in body temperatures in the control and in transit periods were made and the excursions in DBT associated with specific events, and the prevailing thermal micro-environment during these periods were assessed.

#### Deep body temperatures in transit and control periods

The mean body temperatures of the sentinel pigs for the entire travel period for all the journeys are presented in Table 24 and these values may be compared with those presented in Table 25 for a comparable control period for the same pigs. The most striking feature is perhaps the absence of any major differences between the control and treatment (transported) pigs on any journey. The means do not differ by more than 0.3°C on any of the shipments. There are slightly larger differences in the mean lowest temperatures observed in the two groups but these are still less than the daily variations in DBT observed in normal pigs. A comparison of the maxima observed reveals that the highest DBT occurred in transit on Journey 23 (40.6°C) but maxima of 40.4 and 40.5°C were seen in pigs during the control period in the home pens. Even on the basis of this initial analysis, it can be concluded that on the journeys studied, the imposed thermal loads did not result in a heat stress causing DBT to rise significantly.

It is pertinent at this point to introduce the concepts of homeostatic success and homeostatic effort. As animals employ adaptive responses to minimise changes in a controlled variable, such as DBT, then it is necessary to assess any imposed stress in terms of how much a controlled variable changes in response to a quantified challenge, and how hard the animal works (e.g. by panting, sweating, shivering, behavioural thermoregulation, etc.) in order to attempt to control the variable. In pigs in transit, behavioural thermoregulation including postural changes, exploitation of air flow and increased drinking (when water is available), will all contribute to the effort to maintain a constant DBT. These responses will be accompanied by panting and an overall increased evaporative heat loss during exposure to elevated ambient temperatures and thermal loads (e.g. high temperature plus high humidity). These factors must be considered when interpreting the DBT data described above. It is suggested that although the different thermal micro-environments experienced on the different journeys may have placed different demands upon thermoregulatory responses of the pigs, by varying the intensity of such adaptive responses, the pigs were able to maintain control of DBT within fairly narrow bands. These findings and suggestions are consistent with the body temperature data obtained from the spot or point measurement techniques.

The lack of marked effects upon the DBT resulting from exposure to any of the thermal micro-environments on the seven journeys was confirmed by examining the data presented in Figures 37-43. In these figures, the DBT values measured in transit have been plotted against the time for the whole journey period from the UK to Spain. The range of DBT values for the same groups of pigs for the corresponding control periods in the home pens have been superimposed on these data to indicate the expected range of normal DBT values. For all the journeys there is a common response pattern in transit. The DBT tends to fall during the first stage of journey and during the ferry crossing to France from the UK. The DBT rises sharply upon arrival at the control post, probably as a result of unloading, introduction to a novel environment and the provision of feed. The latter factor appears to be responsible for a second rise in DBT towards the end of the lairage period at the control post, immediately prior to reloading for the final phase of the journey. The highest DBT values observed in the study were consistently during the lairage/control post period (regardless of journey month), and the only excursions in DBT outside the control ranges occurred during this period on journeys 23 and 24. The body temperature was generally higher throughout the time at the control post when compared to other phases of the journey. During the early and late journeys (J21, 22 and 27), the body temperature values during the last phase of the journey (last 22 hours) tended to return to values similar to those observed in the first journey phase and within or below the normal control range. On journey 23, the last phase DBT values fell within (almost entirely) the control range but on journeys 24, 25 and 26 the values were in the upper "normal" range or marginally above the upper limit of that range. The higher DBT values tended to occur towards the last few hours of the journey. It is apparent that no major derangements in the control of DBT occurred on these journeys, despite the imposition of significant thermal loads on the high summer shipments.

As with the thermal micro-environment data, it was thought pertinent to analyse the final stage of the journey (last 22 hours), despite the above findings and observations, as this period was associated with exposure to the highest heat loads. The mean DBT values for the last 22 hours of the journey and the corresponding and comparable 22-hour control period are presented in Tables 26 and 27 respectively, and the data are also plotted in Figure 44. As reported for the whole journey there are no major differences in the values (mean, maximum or minimum) associated with transportation or the associated thermal micro-environments. Inspection of Figure 44 reveals that the DBT values on all journeys tended to increase throughout the final 22 hours of the journey. This response was gradual for about half the period after which larger increases occurred. This latter period is associated with travel through northern Spain and the central plain. Later there appeared to be a small decrease in body temperature followed by more rapid increase in the last 1-2 hours of travel. This corresponded to a period when vehicle speed was probably reduced on more rural and smaller roads and the ambient temperatures had increased as the vehicle travelled through southern Spain (Andalucía).

The DBT values in the last 11-12 hours of the journeys were highest on journey 25 and can be ranked in descending order as 25>24>26>27>21>22>23. The most marked increases during the final transport period were greatest in journeys 25, 21, 23 and 27, with much smaller changes observed in journeys 22 and 26. Whilst there are no obvious correlations between the imposed thermal loads across all seven journeys and these DBT responses, it does appear that the most likely journeys on which the body temperature changes might reflect a degree of heat stress are journeys 24 and 25. These correspond to the top of the rankings derived from the spot measures of body temperature and the physical descriptions of the on-board thermal micro-environment. This is confirmed by the data presented in Table 28, which contains the body temperature scores for the final 22 hours of the journey and the resultant ranking. The scores ranged from -3 to +4 (including zero) depending upon the magnitude of the change in mean body temperature when compared to the normal range determined in this study. Thus the largest decreases in DBT would score -3 and the largest increases +4. A score of 0 was ascribed to any values in the normal range. The scores and rankings presented in Table 28 predict that journeys 24 and 25 would represent the highest risk of heat stress, but journey 23 the lowest. The latter finding is at odds with others reported above and is discussed below.

When the data are presented graphically (Figure 45) and corrected for sign, then the risk of heat stress leading to a change in body temperatures in journeys 24 and 25 is emphasised and the risk of changes in DBT is elevated in journey 23. However, this includes a number of apparently low DBT values, which are reflected in the overall response curves in Figure 44. Thus, as the mean body temperature was low compared to several of the other journeys throughout the last 22 hours, then the significant rise in DBT occurring in the last 1-2 hours of transport is not reflected in the mean values employed in the scoring and ranking procedure.

All of these findings confirm that it is necessary to use a number of methods of assessment of the risk and extent of the imposition of thermal stress in transit. In addition, great care is required in the interpretation of the experimental findings which, when reduced to simple, single indices of biological response, may not fully and accurately describe the extent or nature of these responses in the animals.

#### Summary of the data from continuous measurement of DBT in transit

There were no major changes induced in DBT recorded on the surgically implanted data logger system. The range and mean values of DBT were not different from those during a comparable control period in the home

pens. There was a tendency for DBT to increase during the last 22 hours of the journeys (in all cases) and a sharper increase occurred in five of the seven journeys during the last 1-2 hours as the vehicle travelled more slowly on rural roads in hotter conditions. Combined with the preceding data on point measurements and the data describing the physical or thermal micro-environments on the vehicles, it was concluded that journeys 23, 24, and 25 imposed conditions that may cause a degree of heat stress, and that journeys 22 and 26 posed some problems although less than the hotter journeys. Journeys 21 and 27 undertaken in the cooler months of the early summer/autumn did not constitute any threat to the animals' well-being and the conditions did not impose untoward thermal loads in transit.

## 4.4.2 Behavioural measures – animal responses to transport and thermal micro-environment

The behavioural measures employed in the study and undertaken at the end of transport period in the lairage of the slaughterhouse provide extremely valuable information on how the animals had responded to transportation and the conditions in transit. The behavioural indices can be considered in isolation as descriptors of the animals' state at the end of the journey, and to ascertain the animals' priorities in terms of requirements for homeostasis and recovery at the end of travel. Also the behavioural measures can be considered in conjunction with the physiological (and physical environment) measures in order to determine the overall stress imposed by each journey and the associated thermal conditions. Indeed the behaviours can be considered to reflect the homeostatic effort in transit to complement the indices of homeostatic success from the body temperature measures. Of course, the behaviours also allow assessment of the fatigue in the animals post-transport and their general capacity for activity.

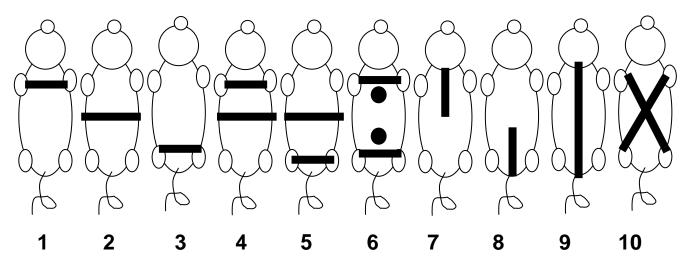
#### Protocol and ethogram for the continuous collection of behavioural data from pigs post transport

Behaviour was recorded live using continuous sampling on pre-designated groups of pigs using the Psion 'Work About' hand held computer running the Observer Package behavioural software 3.0 (Noldus). At the same time a digital video camera (Canon XM2) was mounted on a bracket (Manfrotto magic arm) above each designated behaviour pen to give a clear view of the pigs and their behaviour. Tapes were changed every hour and cameras were powered by long-life batteries lasting over 4 hours.

#### Transport and Identifying Pigs

- Pigs were loaded onto the truck in eight pens of 10 pigs.
- Pens 5 and 6 on truck (middle tier back two pens) contained implanted and chipped animals (sentinel animals) that were separated off on arrival at destination for physiological data sampling (see physiological data collection protocol). These pigs could not be observed for behavioural data because they underwent over 2 hours of physiological sampling post-transport, and therefore uninterrupted free access to drink, rest and recover was not possible.
- Two pens of non-implanted pigs were designated as animals for behavioural sampling. These pigs will came from pens 8 and 9 (bottom tier back two pens) which were immediately below the implanted animals.
- The behaviour pigs were spray marked immediately on entering the lairage pen on arrival and before any
  water drinkers or showers are turned on. Pigs were marked so as to be clearly visible to the human observer
  and also the digital video camera.

#### Pig Spray Mark System



#### 4.4.2.1 Live Behavioural Recording

#### **Observers**

- For each journey two people were designated to watch two pens of 10 pigs (1 pen each) giving a total of 20 pigs behaviourally sampled per trip.
- Observers were pretrained in the ethogram, protocol and use of the 'Psion Work Abouts' via pilot studies on farm and video analysis and proved to be consitent over time, and were tested for Quality Assurance purposes in inter-observer reliability measures and analysis.
- It was not possible to do inter-observer reliablity on a live behaviour sample therefore the video footage was employed to do both inter- and intra-observer reliability.
- Observers were able to clearly see the pigs in a comfortable position without influencing the behaviour of the pigs. Therefore all observations took place from a high gantry walkway above the pigs.

#### <u>Sampling</u>

- Behaviours were recorded continuously for 3 hours post-transport on each pen of 10 pigs simultaneously, effectively resulting in 10 focal animals per pen. Three hours was chosen as this was the time (from previous studies and observations) by which pigs had stopped drinking and had begun to lie down, if they felt the need.
- Sampling began as soon as the pigs had been spray marked and the overhead shower was turned on. This
  was within 5 minutes from unloading from the truck pen.
- Digital video camera tapes were changed every hour. Data from the video was analysed on completion of all the studies.
- Observers completed "Pig Behaviour Sheets" on commencement of the behaviour sampling and used the sheet throughout the sampling period for any notes, comments or errors made.

#### Behavioural Ethogram

After a pilot project at SAC's Easter Howgate Pig Unit, an ethogram was subsequently adapted by taking out some of the behaviours that were too difficult to monitor, e.g. oral manipulation and panting. Feeding was not included in the final ethogram as the pigs were not fed in lairage.

Due to the nature of the study, behaviours were assessed that might indicate how the pigs coped with transport, changes in temperature and fatigue. Close attention was paid to posture changes and drinking behaviour.

In Table (29) is an ethogram adapted from several publications and in conjunction with colleagues at SAC.

#### 4.4.2.2 Video analysis

By recording the pigs on video at the same time as live observations it was possible to record additional behaviours and coping strategies of pigs that are too difficult to record live on 10 animals simultaneously. Observers could concentrate all their efforts on drinking behaviour and posture changes and the camera recorded the shower usage and locomotive activity of the pigs.

#### Behavioural Ethogram on return to the UK (video recordings)

Due to the nature of the study the focus was on behaviours that might indicate how the pigs coped with transport, changes in temperature and fatigue. The main focus was upon posture changes and activity behaviour. A different ethogram (Table 30) can be adopted for video, as it can be stopped and re-watched again to be sure the correct behaviour is recorded.

## 4.4.3 Analysis of live and video observations

#### Live observation

The data files from the 'Work Abouts' were downloaded after each journey onto the project laptop using Noldus Observer 5 software. Any errors were corrected and the files were backed up for analysis in the UK.

#### Video observation

A M.Sc student has been trained in the use of the ethogram and software and pig behaviour. The video files are currently being watched on a PC through Noldus Observer 5 software and being subjected to full analysis. Although not part of the original proposal, the additional video analysis is considered to be very valuable and when completed, in late 2009, it will be reported to Defra in a supplementary document.

#### Additional recordings

The implanted pigs were also filmed in their home pen environment before transportation. They were filmed in their stable groups using Panasonic CCTV cameras mounted on Manfrotto magic arms above the pen and leading back to a Panasonic Timelapse VCR. Recordings were made for a clear 24-hour period running consecutively for 3 days in order to provide an uninterrupted period for 'normal' pig behaviour. These pigs were not spray marked because they were identified with their ear tag number on their backs for physiological data sampling, and because the pigs are sprayed with specific colour code scheme for the transport. These videos have yet to be watched and scored (see above).

#### Statistical analysis of live behavioural observations

Data were extracted from the observation files collected from the 'Work About' using analysis software in The Observer. These data were then exported to Microsoft Excel where all descriptive statistics and graphs were prepared. The means of all durations, frequencies and latencies of all the relevant behaviours were imported into Minitab version 15 and Genstat version 11, where data were checked for normality and, where appropriate, transformed. The duration of drinking (DD), frequency of drinking (FD) and latency to drink (LATD) were not normally distributed and so were logbase<sub>10</sub> transformed prior to analysis of variance for both the whole observation period and separate one hour observation bins. Duration of resting (DR) was only normalised by logbase<sub>10</sub> transformation for the whole observation period and not the individual hours bins, so no repeated measures ANOVA was conducted on the duration of resting over the individual hours. Standing (not drinking) duration (DS) was transformed to normal distribution using the square of the raw data for both the whole observation period and the individual one hour bins.

Spearman's rank correlations were used to identify relationships between variables for the behavioural measures against those of the environment and deep body temperature. Gentsts (Genstat, 11<sup>th</sup> edition, Lawes Agricultural Trust, VSN International Ltd., Oxford, UK) was used for all statistical analyses.

#### <u>Results</u>

Drinking behaviour was considered to be an important variable because, as described in the physiology section, it reflects the extent of any dehydration due to water deprivation and increased evaporative heat loss due to any heat stress. Drinking behaviour and its various components may therefore be regarded as indirect indices of homeostatic effort. The important features of drinking behaviours are the duration of drinking and drinking bouts, the frequency of drinking and the latency to drink. All the data may be analysed by the hours of observation post-transport and for the total observation period in the lairage at the slaughterhouse. Thus data are given as values

for the first, second and third hours of observation and for the total 3-hour period. The results relating to these measures are presented in Tables 31-33 and Figures 46-50.

It was clear that the duration of drinking was greatest on journeys 23(3) > 24(4) (Table 31 and Figure 46). These differences were apparent in all three consecutive hours of observation and therefore for the total 3-hour period (Table 31 and Figure 46) There was a highly significant effect of journey upon drinking durations within and across hours of observation (p<0.001). The ranking of total drinking duration by journey was 24>23>27>21>25>>22>26. The major significant finding is the apparent differences between J23/24 and the other journeys. Mean total drinking at the end of these journeys was significantly different from all other journeys (p<0.001). Drinking in J23 was 65% higher than in J24 (P<0.001) as indicated in Figure 46. Figure 47 shows that drinking in the first hour after arrival was greatest on journeys 22, 24, 25 and 26, was greatest in the 2<sup>nd</sup> hour on Journey 23, and in the 3<sup>rd</sup> hour of Journeys 21 and 27. These distributions suggest that on the journeys (e.g. J23) where the demand for water was greatest then the drinking was high in the first hour, but increased further in the 2<sup>nd</sup> hour to meet with the demand for rehydration. In most journeys the demand for rehydration was met to a large degree by drinking in the first hour and then tended to decline, and in the coolest journeys (J21 and 27), the demand was apparently low and the requirements were met by low drinking durations in all three post-transport hours.

The frequency of drinking was greatest in journey 23 in total (Table 32; Figures 48 and 49) and was highest in each of the 3 hours of observation. The actual numerical ranking on the basis of drinking frequency is 23>24>25>21>22>27>26. The effect of journey upon frequency of drinking was highly significant in each hour of observation and for total drinking (p<0.001). The mean total drinking frequency was significantly higher in J23 than all the other journeys (P<0.001), where the values did not significantly differ from one another. These data indicate that on journeys where the demand for rehydration may have been increased (e.g. J23 and 24), both duration and frequency of drinking were high, but on journeys where thermal demands were low, (as indicated by the on-board thermal micro-environments such as J21 and 27), there were very frequent bouts of drinking but of short duration, indicating lower total water intake. The mean values for latency to drink on the seven journeys are presented in Table 33 and Figure 50. It may be proposed that, the shorter the latency, the greater the urgency to commence rehydration (degree of thirst). The shortest latencies were observed on journeys 23<25<21, but the values were not significantly different for J24. There was greater latencies (p<0.001) on three journeys (26<22<27) compared to J21, 23 and J25, with neither group being significantly different from J24. There was, however, a large degree of variability on all of the longest latency journeys.

The combination of the drinking duration and frequency data (time x frequency) yielded an overall ranking for post-journey water intake (and therefore requirement for rehydration) of J23 (1340) > J24 (490) > J21 (183) > J25 (168) > J22 (155) > J27 (126) > J26 (91). It appears that the requirement for drinking on the apparently hottest journeys was greatest (J23 and 24), but that other factors may determine or predominate the requirement for water intake after the other journeys, as even the coolest journeys (J21 and 27) were highly ranked on this basis. Overall the drinking data (durations, frequencies and latency values) indicated that the greatest demand for rehydration occurred on J23 and J24 which the previous analyses, both physical and physiological, suggested could involve conditions (especially in the last 22 hours) that would potentially impose a degree of thermal stress and could induce a requirement for increased thermoregulatory (homeostatic) effort. The behavioural findings were consistent with this hypothesis.

Another major concern during long distance transport, and particularly under hot conditions, is the possible induction of fatigue in the transported animals. Perhaps an indication of the degree of fatigue imposed can be assessed by examining the amount of time the pigs spend "resting" after the journey and the amount of time they spend standing (which will include many other "activities", such as drinking). The data can also be compared to and correlated with the durations and frequencies of drinking in order to assess the priority demands for pigs on different journeys and therefore experiencing different thermal environments.

The mean values for durations of resting for each journey are presented in Table 34 and Figure 51 for each hour of observation and in total. The greatest resting, numerically, was seen in the pigs from Journey 24 and the value was highest during the first hour post-transport. However, only the short total resting seen in J23 was significantly different from all the other journeys. There were no significant differences between the other journeys due to high variability. The total amount of time resting was also high in J21 and 26. The time resting was generally low in the first hour and increased in the 2<sup>nd</sup> and 3<sup>rd</sup> hours (Figure 52). The journey ranking in terms of total time spent resting was J24>J21>J26>J25>J22>J27>J23.

From the data presented in Figure 52 it is apparent that the pigs from J24 may be fatigued, as their time resting was very high during the 2<sup>nd</sup> and 3<sup>rd</sup> hours, and was only exceeded in the first hour by the pigs from J21. On this journey (J21) resting was high in all three hours post-transport. The pigs on that journey had devoted a similar amount of time to drinking in all three hours, and this allowed the animals to accommodate a significant water intake and considerable resting in the whole post-journey observation period. The small amount of time devoted to resting in J23 is attributable to the fact that these animals spent a great deal time drinking (and thus standing)

and the two activities (resting and drinking) are mutually exclusive in relation to behavioural observations. When two such activities are analysed in tandem it is necessary to consider the animals' priorities and any "trade offs" they may make. Thus it may be that when rehydration is not imperative, and is therefore not the major priority; animals may rest for longer in the absence of significant fatigue. Alternatively if dehydration is a threat to wellbeing the animals may drink extensively at the expense of resting, which then might become a priority when rehydration is adequate. Obviously other intermediate permutations are possible. These factors emphasise the necessity to take a holistic approach when characterising journeys in relation to the risk of stress and poor welfare, as all the animals' responses and apparent priorities must be considered.

The mean values for the time spent standing are presented for each journey in Table 35 and Figures 53-56. It is important to stress that whilst times spent standing and resting are mutually exclusive, the parameters standing and drinking are not. Thus the times spent standing not drinking and total time spent standing have been determined separately. The data are calculated as the time spent standing in each hour and also across the total three-hour period. Inspection of the data indicated that there are only small differences in the time devoted to standing by the pigs across all the journeys (Table 35), although the effect of journey was statistically significant for both total standing and standing not drinking. Small differences occur on J24, when the animals spent a great deal of time resting, and thus total time standing and standing not drinking are reduced (see Figures 53 and 55). A similar, but smaller, effect was seen in J21.

The total time spent standing on J23 appeared slightly longer than on the other journeys due to the much longer time spent drinking (see Figures 53 and 55). It is clear, however, that only the decrease in standing time (total and not drinking) seen in J24 was statistically significant (P<0.001). The analysis by hour of the time spent standing not drinking (Figure 54) reflects the fact that more animals rested in the last hour of observation, and thus the standing time declined after the first 2 hours. The exceptions were J22 and 23 in which standing not drinking was maintained in the final hour. The mean total standing periods analysed by hour (Figure 56) show the overall pattern as described above. Thus the pigs were standing a great deal in the first hour, when drinking and other standing were maximal. Total time standing tended to decline during the 2<sup>nd</sup> and 3<sup>rd</sup> hours of observation in all but J22. It must be emphasised that the range of times standing across the whole study actually varied little, even across consecutive hours and variability within a mean estimate was high.

It is proposed that drinking and resting behaviours provide the most important insights in to the animal postjourney responses and conditions, and may be better indices of any homeostatic effort resulting from thermoregulatory demands imposed by the transport thermal environment. This proposal is supported by the data presented in Table 36 and Figure 57. Here, the proportions of time allocated by the pigs to each of the main activities analysed are compared. The percentages of time devoted to drinking, standing (not drinking) and resting are given for all seven journeys. The most striking features are that as reported above, on J23 and 24 much more time was occupied by drinking (18.6 and 11.4% respectively) across the whole 3-hour observation period. It is proposed that this indicates more water loss in transit on these journeys, possibly resulting from an increased demand for evaporative heat loss (hotter journeys?). On J24, a large proportion of the post-transport time (24.2%) was spent resting and it is suggested that this may reflect a high degree of transport-induced fatigue. On journey 21 a large proportion of time was spent resting and it is proposed that the other demands for drinking were not as urgent, and being equally distributed over the whole (3 hour) lairage period allowed time for additional resting in each hour. These patterns are expressed graphically in Figure 57.

Based upon a consideration of all the behavioural variables quantified and analysed, it is possible to rank the journeys in terms of thermal stress and the behavioural responses invoked by the thermal loads in transit. When drinking alone is considered it may be proposed that J23 and J24 may be considered to be classified as hot, requiring high evaporative water loss and therefore elevated water intake upon arrival. J22, 25 and 26 can be considered intermediate and thus classified as warm, whereas J21 and J27 impose fewer demands on evaporative heat loss and hydration state and thus may be considered cool. These data and the categorisations are presented graphically in Figure 58.

When the requirement to rest is incorporated it appears that the categorisation remains essentially similar, as the demands in J24 resulting from fatigue emphasise the stressful nature of that journey.

## 4.5 Journey categorisation by physical, physiological and behavioural means

On the basis of the "on-board" thermal conditions (presented in section 3) it was proposed that the categorisation of the journeys should be that Journeys 23 and 24 (and 22?) should be designated as hot. Journeys (22) 25 and 26 are classified as warm and Journeys 21 and 27 as cool. It was suggested that the characterised thermal conditions classified in this way reflected the risk of the imposition of thermal stress by the temperatures, water vapour densities and enthalpies in the transport space within the vehicle. This was particularly appropriate when

the last 22 hours (final phase of the journey) was considered. It was clear that the highest heat loads were imposed during this period, particularly in the last few hours prior to arrival at the slaughterhouse.

Based on the physiological measures and responses (in subsection 2) it was suggested that journeys 23, 24, and 25 may impose conditions that may cause a degree of heat stress and that journeys 22 and 26 may pose some problems although less than the hotter journeys. The conditions on journeys 21 and 27 undertaken in the cooler months of the summer/autumn may not constitute any threat to the animals' well-being, and the conditions may not impose untoward thermal loads in transit. Again, when the final 22 hours of the journey were considered it is proposed that the thermal loads in this journey phase perhaps imposed the greatest stress on the pigs and were responsible for the physiological responses observed.

In the behavioural subsection (4.4.2) it has been proposed that J23 and J24 may be classified as hot, requiring high evaporative water loss and therefore elevated water intake upon arrival. J 22, 25 and 26 may be classified as warm, and it is proposed that J27 imposes lower demands on evaporative heat loss and hydration state and thus may be considered cooler. On journey 21, despite some anomalous results, it can still be defined as lower thermal stress transport. This categorisation is supported by the other behavioural analyses. It is suggested that the thermal conditions on the vehicle during the last phase of the journey (last 22 hours) may have had the major influence upon post-transportation behaviours.

There is a high degree of consistency between the different methods of classification or categorisation between the separate approaches. It appears that the journeys undertaken in July (J23 and 24) were associated with the biggest risk of thermal stress and the expression of physiological and behavioural responses associated with the elevated heat loads. Journeys undertaken in June and August (J22, 25 and 26) may impose some degree of heat stress and caution should be exercised when transporting the animals under these conditions. The journeys undertaken in early June and in October (J21 and J27) when ambient temperatures were lower imposed little, if any, thermal stress and consequently constituted little risk to the well being or welfare of the pigs.

In order to provide some support for these suggestions and predictions, the data from each subsection (physical, physiological and behavioural) of section 3 of this report were subject to correlation analysis to determine if any significant relationships between the many and different variables determined could be established.

# 4.6 Correlations of parameters from the physical (thermal environment), physiological and behavioural components of the study

The correlation matrices are presented in Figures 59 and 60. The first matrix (Figure 59) contains the analyses for the physiological variables, body weight changes, lesion score differences and packed cell volumes in relation to the vehicle mean temperatures and enthalpies calculated over the last 22 hours of the journey. The second matrix (Figure 60) contains all the corresponding behavioural and environmental data.

It is apparent that there are few obvious correlations within the physiological data. The high correlation between the physical thermal measures or indices is due to the methods of derivation. The significant correlations between PCV and thermal load (and a negative correlation with DBT) are unlikely to reflect dehydration 18 hours after arrival at the slaughterhouse and after prolonged drinking. These observations are difficult to explain.

The change in RFID chip temperature was correlated with the mean enthalpy during the last 22 hours of the journey and may reflect a genuine effect. As might be predicted, there was a correlation between change in chip temperature and change in surface temperature and change in rectal temperature. The apparent negative relationship between DBT and environmental temperature and enthalpy is difficult to reconcile with the findings relating to the effects of the last 22 hours on continuous measures of DBT described above. In order to successfully develop predictive models for physiological responses in the future, a more comprehensive correlation matrix should be created, including more physiological response indices and variables. The behavioural indices should also be included in these models.

In the behavioural correlation matrix many more significant correlations were identified. The most important are the positive correlations between the mean vehicle temperatures (and enthalpies) and the durations and frequencies of drinking in the first hour post-transport, drinking durations in the second hours post-transport, total drinking over 3 hours, drinking frequency in the third hour and total observed drinking frequency. These correlations support the hypotheses relating thermal load to dehydration and increased requirement for water intake post-transport at the higher thermal loads. The absence of significant correlations between DBT and the behavioural measures is probably attributable to the mechanisms discussed above. The demand for evaporative heat exchange imposed by the higher thermal loads has been met by panting and a degree of dehydration (reflected in post-transport drinking). This is an indication of the expended thermoregulatory or homeostatic

effort. As homeostatic success is high, i.e. DBT is controlled within a narrow range, then a lack of response in this variable precludes a correlation with behavioural indices. As with the physiological models, a more comprehensive correlation matrix is to be derived (at SAC, outwith the current project) to facilitate further model development.

## 5. Overall summary, conclusions and recommendations

- The effects of each journey on the animals may be assessed by:-
  - Body temperature responses
  - o Drinking behaviour / hydration state
  - o Resting behaviour / fatigue
- These indices have been integrated and correlated with the physical or thermal categorisation of each journey.
- There is a high degree of consistency between the different methods of categorisation between the separate approaches.
- Integration of the physiological and behavioural data indicates that most adaptive responses occur in Journeys 23, 24 and 25, and it is suggested that some degree of transport stress was associated with these journeys (conditions?).
- Thermal conditions on the vehicle during the last phase of the journey (last 22 hours) had the major influence on post-transportation behaviours and physiology of the pigs.
- Journeys 21, 22 and 27 (conditions) did not impose excessive transport stress upon the pigs.
- The journeys covered a typical range of thermal conditions and transport micro-environments for Southern Europe.
- The thermal conditions included journeys that might be classified as "mild", "warm" and "hot".
- A number of physiological and behavioural measures and analyses were correlated with the vehicle thermal micro-environment.
- On none of the journeys was severe thermal stress identified.
- All pigs appeared in good health and condition upon arrival.
- The deep body temperature did not exhibit any major excursions outside the normal ranges recorded in the home pen. The only exceptions correlated with feeding and arousal during lairage.
- Journeys that were scored highly on elevated temperatures and enthalpy were associated with increases in both physiological and behavioural indices of stress.
- Thus journeys 23 and 24 (25?) may have been associated with some degree of thermal stress (but not severe).
- It appears that the journeys undertaken in July (J23 and 24) were associated with the biggest risk of thermal stress and the expression of physiological and behavioural responses associated with the elevated heat loads. Journeys undertaken in June and August (J22, 25 and 26) also imposed some degree of heat stress and caution should be exercised when transporting the animals under these conditions.
- These journeys had periods when the conditions were in the upper ranges for temperature (30°-35°C) currently prescribed in the Regulation EC 1/2005.
- The journeys undertaken in early June and in October (J21 and J27) when ambient temperatures were less than in high summer imposed little, if any, thermal stress and consequently constituted little risk to the well being or welfare of the pigs.

- Whilst journeys undertaken in conditions close to or at the limits of temperature prescribed in the current regulation were associated with some physiological adaptive responses, these did not constitute a major threat to the welfare of the animals.
- It is proposed that the role of humidity or water vapour density should be examined in future studies in relation to temperature limits.
- Enthalpy may a useful practical index/predictor of thermal stress.
- The results suggest that if transportation is undertaken in a manner consistent with current legislation, on appropriate vehicles and with high standards of personnel and practice, there is little threat to the welfare of the pigs even in relatively hot conditions (and under conditions typical of Southern Europe in summer).
- It is suggested that the prescribed temperature limits are revisited and reviewed in order that future legislation may take full cognizance of the animals' physiological and behavioural responses and sound scientifically-based definitions of acceptable thermal envelopes for transportation.

Current legislation (EC 1/2005) contains an upper limit of  $30^{\circ}C \pm 5^{\circ}C$  for the transportation of all livestock on journeys of over 8 hours duration. It is apparent from measures of temperature and relative humidity in southern Europe that heat loads in this range will frequently be encountered in commercial practice. The current Regulation does not include reference to the duration of exposure to elevated thermal loads that should be permitted or prohibited. This is a serious omission. The current study has demonstrated that on higher standard vehicles with adequate ventilation and a high standard of animal care, temperatures of around  $30^{\circ}C$  need not necessarily impose undue heat stress upon breeder pigs in transit. It is therefore suggested that to provide a sound scientific basis for any revision of the Regulation, future research studies should focus upon the effects upon animals (e.g. breeder pigs) of temperatures in the range  $28^{\circ}$  to  $36^{\circ}C$  (with a range of appropriate relative humidities) for appropriate ranges of periods of exposure. This will provide indications of what temperature/humidity combinations would be acceptable for each of the exposure periods. Only through such an approach will it be possible to ensure the welfare of animals in transit in hot weather through the provision of precise limits for imposed thermal loads and durations of exposure. These issues should be addressed through both modelling studies and further concomitant field trials and it is recommended that this be a major research priority.

In addition there are other factors that influence animals' responses to thermal conditions, including during transportation and these must be given due consideration. Previous experimental and practical studies (see Defra project AW0815) have emphasised the importance of the phenomena of thermal adaptation and acclimatisation in the determination of the responses of animals (pigs) to the transport thermal microenvironment. Both adaptation and acclimatisation are well recognised in pigs (Mitchell and Kettlewell 2004, Kettlewell and Mitchell, 2005, Mitchell and Kettlewell, 2005; Renaudeau et al 2005, 2006 & 2008). The thermal history and genetic background of animals clearly have a profound influence on their subsequent response to thermal challenge. Well-adapted pigs in Mediterranean climates may have a much greater heat tolerance and thus the acceptable upper limit for temperature exposure may be higher for these animals. Similar arguments may be applied to cold exposure in more northerly climes. It is difficult to conceive of how this might be incorporated in to pan-European legislation as acclimatisation will vary with geographical location, time of year and age of the animals but will be a major determinant of the effects of thermal loads in transit upon the welfare of the animals.

Overall, however, it is suggested, therefore, that a modelling approach should be applied in future to all transport conditions to allow the definition of safe thermal envelopes that will include temperature, water vapour density of humidity and air movement but will take full cognizance of the physiological state of the animal including adaption and acclimatisation.

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## References to published material

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

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