



End of Project Report

THE WELFARE OF ANIMALS TRANSPORTED FROM IRELAND TO ITALY

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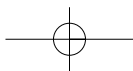
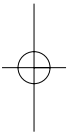
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DEDICATION

*My effort in the conduct of this research is dedicated
to the loving memory of
my Father,*

Patrick Earley

Who died on October 21st, 2002

*His example of hard work, perseverance,
faith and great love will always inspire me.*

*A silent thought, a quiet prayer,
For a special person in God's care*

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I. SUMMARY

Twenty-six weanling continental x beef bulls (mean liveweight 414kg \pm 55.8kg) were transported from Ireland to Italy on the 18th of October 2002, on a roll-on roll-off ferry (RO-RO), and onwards by road for 3-hours to a French lairage (Fougeres), rested for 24 hours at a staging post and taken by road on an 18-hour journey through France to a feedlot in Italy.

Prior to transport (Day 0) the median rectal body temperature for control animals was 39.1 \pm 0.55°C and ranged from 38.2 to 40.4°C. The median rectal body temperatures of the animals assigned to transport were 39.3 \pm 0.60°C and ranged from 38.2 to 40.6°C, respectively.

On day 10 of the study, 5 days after arrival in Italy, an outbreak of pneumonia occurred in the animals in the feedlot in Italy. One animal died and all animals were vaccinated (Risposal and Immuresp) against respiratory syncytial virus (RSV) and *Pasteurella* (Presponse). In Italy, animals showing signs of pneumonia were injected with the antibiotics Amoxil (long acting) and Micotil. Two bulls died on the control farm in Ireland following an outbreak of respiratory disease which occurred after day 11 of the study.

There were no differences in liveweight between the control and transported groups before the journey. The mean liveweight of the transported group decreased by 7.0% by the time of arrival at the staging post in Fougeres, France. The mean daily liveweight gain (kg/day \pm sem) for the control and transported treatments were 0.24 \pm 0.16kg compared with 0.43 \pm 0.38kg from October 17th to November 26th; 0.61 \pm 0.17 compared with 1.59 \pm 0.12kg from the 28th of October to the 26th of November. There was no significant difference in final liveweight between control and transported animals, however the mean liveweight gain/day for the transported animals was significantly greater ($P < 0.047$) than controls. The finishing period from day 40 to slaughter were 193.9 \pm 20.82 days for controls and 160.7 \pm 43.16 days for transported animals, respectively. Carcass weight and kill out percentage was significantly greater in transported animals.

There was no significant difference in plasma protein or globulin concentrations prior to transport between control and transported animals. Following transport, protein concentrations were significantly higher at samples 2 to 9 post-transport and Control animals had significantly higher protein concentrations for the corresponding time points. There was a significant increase in albumin concentrations following the transport journey to France, and concentrations remained elevated at 12 and 24 hours after arrival and up to day 11 post transport and concentrations were significantly lower on day 40, compared with pre-transport baseline values. Plasma albumin concentrations were significantly higher in control animals and remained elevated up to day 11. By day 40 of the study, control animals had significantly lower albumin concentrations when compared to pre-transport values.

There was no significant difference in red blood cell (RBC) numbers prior to transport between control and transported animals. RBC numbers were significantly increased at arrival in France (sample 2), 12 hours after arrival (sample 3), at arrival in Italy (sample 5) and significantly lower on day 40 (sample 9) of the study. Control animals had significantly higher concentrations at sample 3 and 4, and 6 (day 7 of the study) and lower number at sample 8 and 9 (day 11 and day 40). There was no significant difference in plasma haemoglobin concentrations prior to transport between control and transported animals. There was a significant increase in haemoglobin concentrations following the transport journey to France, and concentrations remained elevated at arrival and 12 hours after arrival in France, and on day 5 of the study (arrival in Italy). Plasma haemoglobin concentrations were significantly higher in control animals and remained elevated at sample 3, 4 and 6 (days 3, 4 and 7 of the study) and significantly lower at sample 8 and 9 (day 11 and 40 of the study). There was a significant increase in haematocrit percentage at sample 3 (day 3 of the study) and decrease at samples 6 (day 7) and sample 8 (day 11) in transported animals. Control animals had significantly higher haematocrit percentage at sample 3 (day 3) and sample 6 (day 7) and significantly lower values at samples 8 and 9 (day 11 and 40 of the study). There was no significant

difference in white blood cell (WBC) numbers prior to transport between control and transported animals. Following transport, WBC numbers were significantly higher at sample 4 post-transport. Control animals had significantly lower WBC numbers at sample 2. There was no significant difference in lymphocyte or neutrophil percentages prior to transport between control and transported animals. Transported animals had significantly lower lymphocyte percentages at sample 2, (day 2; arrival in France time 0) and sample 4 (day 4 of the study). There was a significant increase in neutrophil % at sample 6 and 7 post transport. Control animals had significantly lower neutrophil percentages at sample 2 and higher percentages at sample 8 (day 11 of the study). There was no significant difference in urea concentrations prior to transport between control and transported animals. Plasma urea concentrations were significantly increased at sample 2 (arrival in France; time 0) and 3 (12 hours after arrival in France), and decreased at samples 4, 6, 8 and 9 post-transport. In control animals, plasma urea concentrations were significantly decreased from sample 2 to sample 8 of the study.

There was no significant difference in BHB concentrations prior to transport between control and transported animals. Following transport, BHB concentrations were significantly increased at sample 2, 3, 4, 5, 6 and 8. Control animals had significantly higher BHB concentrations at samples 2, 3, 4, 5, 6 and 7. There was no significant difference in creatine kinase (CK) concentrations prior to transport between control and transported animals. Following transport, CK concentrations were significantly increased at sample 2, 3, 4, 5 and significantly lower at sample 7, 8 and 9. Control animals had significantly lower CK concentrations at sample 6, 7, 8 and 9. There was no significant difference in plasma NEFA concentrations prior to transport between control and transported animals. Following transport, plasma NEFA concentrations were significantly higher at sample 2 to 8 and lower at sample 9 following transport. Control animals had significantly higher plasma haptoglobin concentrations at samples 2 to 8.

There was no significant difference in plasma glucose concentrations prior to transport between control and transported animals.

Following transport, plasma glucose concentrations were significantly lower at Samples 5, 6, 7, 8, and 9 (days 5 to 9 post-transport).

There was no significant difference in concanavalin-A (CON-A) induced interferon- γ (IFN- γ) or in phytohaemagglutinin-A (PHA) induced interferon- γ (IFN- γ) concentrations prior to transport between control and transported animals. Following transport, CON-A induced IFN- γ concentrations were significantly lower at sample 6 and 7 (days 7 and 9 of the study). Control animals had significantly lower IFN- γ concentrations at sample 6 and 8. PHA-induced IFN- γ concentrations were significantly lower at sample 4 and 7 (days 4 and 9 of the study) following transport. Control animals had significantly lower IFN- γ concentrations at sample 6 (day 7 of the study). There was no significant difference in plasma fibrinogen and haptoglobin concentrations prior to transport between control and transported animals. Following transport, plasma haptoglobin concentrations were significantly higher at sample 2 to 9 following transport. Control animals had significantly higher plasma haptoglobin concentrations at samples 3, 4, 5, 6, 7, and 8. There was a significant increase in fibrinogen concentrations following the transport journey to France, and concentrations remained elevated at 12 and 24 hours after arrival and up to day 40 of the study.

There was no significant difference in serum antibody concentrations for infective bovine rhinotracheitis virus (IBR) prior to or after transport between control and transported animals. There was no significant difference in serum antibody concentrations for the respiratory syncytial virus (RSV) prior to transport or at samples 1, 4, 5, 7 for control and transported animals. Control animals had significantly higher RSV antibody titers at day 40 (sample 9) compared with pre-transport baseline values. Transported animals had significantly higher RSV titers at sample bleed 8 and 9 (days 11 and 40 respectively). There was no significant difference in serum antibody concentrations for the para-influenza-3 (PI-3) virus at samples 1, 4, 5, 7 for control and transported animals. Control and transported animals had significantly higher PI-3 titers at sample bleed 9 (day 40). This sero-conversion in control and transported animals was mainly related to the outbreak of respiratory that occurred in both treatments.

Based on the immunological and physiological measurements made and the behavioural observations the transport journey under the present conditions is not unacceptable from the viewpoint of animal welfare.

The study concluded that transport had no adverse effect on animal welfare based on the physiological, immunological and haematological measurements made.



2. INTRODUCTION

The protection of animals during transport is an important concern of the European Commission. The first Community legislation on the protection of animals during transport, Council Directive 77/489/EC, reflected the relevant 1968 Convention of the Council of Europe. It has since been replaced by the more detailed Council Directive 91/628/EC as amended by Directive 95/29/EC which introduced changes such as the approval of transporters, route plan, as well as loading densities and travelling times limit. Additional legislation reinforcing Directive 91/628/EEC was adopted in 1995, 1997 and 1998. Transportation of livestock is perceived as an acute stressor and involves several potential stressors that result in increase cortisol (Kenny and Tarrant, 1987a, b), altered products of energy and protein metabolism (Todd *et al.* (2000), with associated changes in appetite and growth rate and a challenged immune system (Blecha *et al.*, 1984; Murata *et al.*, 1987) resulting in increased disease susceptibility. Other physical factors such as noise or vibrations; emotional factors, such as unfamiliar environment or social regrouping; and climatic factors, such as temperature, humidity, or oxygen concentration, are also involved.

The overall objective of the present study was to investigate the physiological, haematological and immunological responses of weanling bulls transported to Italy under present EU legislation and to evaluate the implications in terms of animal welfare.



3. OBJECTIVES

1. To make appropriate physiological measurements on the animals to quantify the effect of transport on the degree of stress imposed and the ability of the animals to cope with that stress.
2. To monitor and record the environmental conditions on the vehicle (as normal) thus enabling the heat and moisture production of the animals to be determined.

Study hypothesis: the welfare of animals transported from Ireland to Italy will not be compromised in transit or subsequently as a result of the journey.



4. MATERIALS AND METHODS

Twenty-six weanling continental x beef suckler weanlings (mean \pm s.e. liveweight $414 \pm 55.8\text{kg}$), sourced from 5 different beef suckler farms in Counties Galway and Clare, having been weaned on October 17th were transported by road and sea to Italy.

On the morning of the journey (October 17th, 2002), 26 animals were blood sampled ((day 0; Sample 1) by jugular venipuncture to provide baseline physiological values on the farms of origin)) and weighed.

The twenty-six animals were taken to a local farm Co. Galway and lairaged overnight, feed and water were freely available. On the morning of the journey, the animals were loaded and transported by road to Sandyford, Co. Dublin, unloaded for veterinary inspection, and loaded onto the transporter at 16:30h, into 4 fan ventilated pens at a stocking density of (1.2m^2) per animal, on an air suspension double deck articulated transporter and transported by road to Rosslare, Co. Wexford.

The pens on the transporter were bedded with straw and water was available through nipple drinkers. The ferry departed Rosslare at 20:15h and the journey took approximately 23hours.

The average speed during the sailing ranged from 16.5 to 17.0 knots/hr, the wind/force ranged from calm, to Northerly to South-Easterly direction, and the ambient temperature ranged from 8 to 11°C . Twenty-two weanling continental x beef breed bulls ($416 \pm 60\text{kg}$ liveweight) were weaned at the same time as the transported animals and remained on the control farms and were blood sampled and weighed at times corresponding to the transported animals.

Table 1: Experimental protocol for the transport study from Ireland to Italy.

	Pre-transport to France	Departure (Ferry)	Arrival in French lairage	Lairage	Lairage Depart for Italy	Arrival in Italian Feedlot	Feedlot			
	Ireland to France	Ireland time 0	France +12 hr	France +24 hr	France	Italy	Italy			
Date	17th-Oct	18th-Oct	19th-Oct	20th-Oct	21st-Oct	22nd -Oct	24th-Oct	26th-Oct	28th-Oct	26th-Nov
Day of study	0	1	2	3	3	5	7	9	11	40
Sample No.	1	2	3	4	5	6	7	8	9	
	Live weight		Live weight			Live weight			Live weight	Live weight

On arrival in Cherbourg, France on October 19th at 20:15h (local time), the animals were transported by road for 3h to a lairage in Fougères, where they remained for 24 hours. At the lairage, animals were unloaded, and weighed. They were blood sampled immediately on arrival (Day 2; sample 2), and again at 12 hours (Day 3; sample 3) and 24 hours (Day 3; sample 4) after arrival in the lairage by jugular venipuncture into blood collection tubes containing anticoagulant (see Table 1 with experimental protocol of study and dates of blood sampling). Hay and water were freely available in the lairage.

The 18 hour journey from the lairage at Fougères in France to the feedlot at Pinerolo, Italy, involved different road surfaces ranging from motorways to country lanes. To comply with current legislation, animals were rested for one hour on the transporter after the first 14 hours of the journey. On arrival in the Italian feedlot (October 22nd) on Day 5, animals were blood sampled (sample 5) and again on days 7, (sample 6), 9 (sample 7) 11 (sample 8) and 40 (sample 9) of the study. The animals were weighed after unloading at the feedlot in Italy, and on day 11 and 40 of the study.

Animal diets and composition

The animals in Italy were fed an *ad libitum* finishing diet ((Concentrate; dry matter 888g/kg; Oil A 16.8 g/kg; crude protein 200g/kg); (Hay; dry matter (DM) 897g/kg; crude protein 153; dry matter digestibility (DMD) 629; ash 137g/kg DM; ADF 329 g/kg DM; NDF 573 g/kg DM); (Straw; DM 918g/kg; CP 65.7g/kgDM, DMD 533; ash 88.6 g/kgDM; ADF 457 g/kgDM; NDF 766 g/kgDM); (Maize silage; 236 DM; CP 63.8g/kg DM; DMD 349 g/kg DM; Ash 132 g/kg DM; water soluble carbohydrates (WSC) 4.3; Lactate 2.3; Ammonia N (NH₃) 7.2; ADF 452 g/kg DM; NDF 783 g/kg DM).

The control animals remaining on the farm in Ireland were maintained on an *ad libitum* silage diet and concentrates (2kg/head) (Concentrate; DM 861g/kg; Oil A 13.3g/kg; Crude protein 141 g/kg DM); (Silage; DM 167g/kg; Crude protein 171 g/kg DM; DMD 653 g/kg DM; Ash 76.3 g/kg DM; WSC 6.9; Lactate 31.5; NH₃ 77.0; ADF 312 g/kg DM; NDF 549 g/kg DM).

Rectal temperatures were recorded using a digital thermometer (Jorgen Kruise A/S; Model VT-801 BWC Lot No 0701) prior to transportation (day 0) and on days 2, 3, 5, 7, 9, 11 and 40 of the study.

Behaviour

Lying and standing behaviour of the bulls on the transporter were monitored and video-recorded using 460 lines high resolution black-white cameras (Eneo, Germany) with built in 12 watt infra red lighting. The cameras recorded 19–25 frames /sec. Images were transferred to a personal computer using a multiplex card manufactured by CCTV (UK) (www.cctvsoftware.com).



5. PHYSIOLOGICAL, HAEMATOLOGICAL AND IMMUNOLOGICAL VARIABLES.

Blood samples (Samples 1..9) were collected by jugular venipuncture, into heparinised tubes, centrifuged and the plasma separated for subsequent analysis of cortisol, glucose, lactate, free fatty acids, β -hydroxy butyrate, urea, total protein, albumin, creatine phosphokinase (CK), and the acute phase proteins (fibrinogen and haptoglobin). Blood samples for interferon- γ determination were also collected by jugular venipuncture into aseptic vacutainer tubes containing lithium heparin and the stimulated lymphocyte production of interferon- γ in response to keyhole limpet haemocyanin (KLH) and Concanavalin-A (Con-A), was determined following whole blood culture of heparinised samples, using an ELISA procedure (CSL, Biosciences, Parkville, Victoria, Australia).

The haematological variables (red blood cell number (RBC), haemoglobin (Hb), haematocrit % (or packed cell volume) (PCV), total white blood cell (WBC) numbers, % lymphocytes, % monocytes) were determined in unclotted (K_3 -EDTA) whole blood samples using an electronic particle hematology analysers (CellDyn 3500 Analyser (Ireland), Technicon HI, manufactured by Bayer (Italy), Cheryp-Gaillot French laboratory, PENTRA500 apparatus (ABX company). Plasma haptoglobin concentrations were measured by determining the haemoglobin-binding capacity using a biochemical autoanalyser. Fibrinogen concentrations were measured using a commercial biochemical assay kit (Boehringer Mannheim, Germany). All other physiological measurements were made using Randox assay procedures. Bodyweight, rectal temperature and blood haematology (WBC numbers) were measured as general indicators of health and the ability of animals to cope with transport.

The ambient temperature and relative humidity during transport and in the housing environments in Ireland and Italy were recorded continuously using TinyTalk dataloggers (Radionics, Dublin, Ireland). Environmental measurements on the transporter including carbon

dioxide (CO₂) (ppm), relative humidity (RH), temperature (°C), air velocity (m/s) and vapour density (td°C) were recorded continuously during transit using QRae and logging systems.



6. STATISTICAL ANALYSIS

The physiological, haematological and physiological measurements taken before and after transport were analysed by repeated measures analysis of variance with journey time as the factor. The first sample (Day 0; sample 1) was used as a covariate in this analysis. SAS/STATISTIC® software was used to analyse the data for the study. Pre-planned, matched pair t-tests, to detect changes over time were made using PROC MEANS, the null hypothesis being that the mean difference between selected time points was equal to zero. The PROC GLM repeated measures option was used to test the effects of treatment while controlling for time effects. Analysis was performed on the rank scores of variables that failed the test for normality.

7. RESULTS AND DISCUSSION

7.1 Environmental conditions

The environmental conditions during transport from loading of the bulls in Galway, to Clane by road for veterinary inspection, by sea journey to France, un-loading in France, loading in France and onwards by road to Italy are summarised in Table 2. The values are presented as mean with minimum and maximum values recorded for each parameter. The ambient temperature and relative humidity outside the transporter is also presented for the different stages of the journey.

Table 2

Journey stage	Transporter			Ambient			
	CO ₂ ppm	RH %	Temperature °C	Air velocity m/s	Vapour density td °C	RH % °C	Temperature °C
Galway to Clane by road	0.065	84.9	8.5	0.887	5.99	86.4	9.1
Clane Stationary – on the transporter	0.04–0.16	58.1–99.9	3–15.6	0.01–2.54	1.2–13.0	68–100.9	6.2–11.7
Clane to Rosslare by road	0.08	67.4	13.9	0.11	7.98	68.1	10.7
Rosslare to Cherbourg by ferry	0.04–0.12	54.8–84.7	10.1–15.5	0.01–0.36	3.4–11.2	67–69.4	10.6–11.0
Cherbourg to Fougères by road	0.050	63.7	10.9	0.60	4.25	62.3	9.0
Fougères to Lairage 24 hour rest period	0.04–0.12	54.9–84.0	8.2–13.6	0.06–1.28	2.2–8.4	55.6–68.4	6.9–10.6
Lairage 24 hour rest period	0.09	58.8	16.99	0.14	8.80	59.6	6.97
Lairage 24 hour rest period	0.04–0.17	46.8–86.9	9.5–20.1	0.01–2.05	3.5–14.5	52.0–68.9	2.7–12.4
Lairage 24 hour rest period	0.05	75.5	10.3	1.02	6.13	69.2	7.8
Lairage 24 hour rest period	0.04–0.10	68.5–88.8	8.6–13.5	0.0–2.7	4.1–10.2	65.5–73.9	7.3–8.4
Lairage 24 hour rest period	–	–	–	–	–	89.5	8.0
Lairage 24 hour rest period						64.6–100.9	6.5–10.6
Lairage 24 hour rest period	0.07	79.3	16.5	0.67	15.2	44.4	12.2
Lairage 24 hour rest period	0.03–0.17	63.2–99.9	8.6–23.2	0.0–3.17	10.5–20.9	5.2–100.9	8.8–14.9

7.2 Feedlot – Ambient temperature and relative humidity

The minimum and maximum relative humidity percentages and temperatures recorded for the feedlots in Ireland and Italy were; Ireland, a mean relative humidity of 75.7 % (63.8–100%) and mean temperature of 10.1°C (2.7–18.4°C) was recorded. In the Italian feedlot, the corresponding values with minimum and maximum values recorded were mean relative humidity of 84.7% (18.2–100%) and mean temperature of 10.2°C (3.9–19.8°C) respectively.

7.3 Behaviour during transport

The behaviour data was obtained from video camera recordings of each of three pens on the transporter (pens 1, 2 and 4). The video tapes were scan sampled every 10 minutes and an observation was made of how many animals were standing and lying down. The transporter went from Galway to Clane, remained in Clane for 3 hours. This was followed by a 3.5 hour journey to Rosslare, before embarking on the ferry for an approx 24-hour journey. The transporter remained at the staging post/lairage in Fougères for 24 hours and was followed by a road journey (18-hours) to Italy.

Table 3: The mean percentage average time that animals spent standing and lying during the journey from Ireland to Italy

Journey stage	Standing	Lying
Galway to Clane by road	93.1	6.9
Clane Stationary – on the transporter	64.0	34.1
Clane to Rosslare by road	67.5	32.5
Rosslare to Cherbourg by ferry	36.5	63.5
Cherbourg to Fougères by road	53.7	44.3
Lairage 24 hour rest period		
Fougères to Italy by road	64.7	35.4

As each journey is of different duration, the data is presented as the average percentage time within each journey period. Total lying and standing (activity) times were obtained from 10-minute scan

samples of each pen (pen) for the different time periods (Journey). The percentage total time (Time) was calculated data was analysed using SAS from the frequency of observed activity multiplied by the number of animals in each pen taking into account the total possible number of observations that could occur.

7.4 Rectal temperature

Prior to transport (Day 0) the median rectal body temperature for control animals was $39.1 \pm 0.55^{\circ}\text{C}$ and ranged from 38.2 to 40.4°C (Table 4). The mean temperatures of the animals assigned to transport were $39.3 \pm 0.60^{\circ}\text{C}$ and ranged from 38.2 to 40.6°C , respectively. The rectal body temperature of transported animals was significantly higher at bleed 5, 7 and lower at bleed 8 compared with control animals, with a significant treatment \times time interaction ($P < 0.0007$).

While body temperature were significantly lower for the transported animals, they were still within the normal clinical range (37.8 – 38.8°C) (Anderson, 1993). In animals, normal cellular function depends on a relatively constant body temperature, which is the sum of heat production (or conservation) and heat loss. This temperature is regulated by a central mechanism within the hypothalamus in the brain which activates both physiological and behavioural activities.

7.5 Liveweight

The changes in liveweight are shown in Tables 5a and 5b and Figure 1. There were no differences between the control and transported groups before the journey. The mean liveweight of the transported group decreased by 7.0% by the time of arrival in Fougères (Day 2). The mean daily liveweight gain ($\text{kg/day} \pm \text{sem}$) for the control and transported treatments were $0.24 \pm 0.16 \text{kg}$ compared with $0.43 \pm 0.38 \text{kg}$ from October 17th to November 26th; 0.61 ± 0.17 compared with $1.59 \pm 0.12 \text{kg}$ from the 28th of October to the 26th of November.

The liveweight performance after day 40 to slaughter, and carcass data for the bulls are presented in Table 5b.

There was no significant difference in final liveweight between control and transported animals, however the mean liveweight gain/day for the transported animals was significantly greater ($P < 0.047$) than controls. The finishing period from day 40 to slaughter were 193.9 ± 20.82 days for controls and 160.7 ± 43.16 days for transported animals, respectively. Carcass weight and kill out percentage was significantly greater in transported animals ($P < 0.0032$).

Table 5a: Liveweight (kg) in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Mean \pm SD with P values.

WEIGHT	Sample 1	Sample 2	Sample 5	Sample 8	Sample 9
Control	416 60.0	411 64.7	409 60.6	406 57.5	424 63.1
Matched Pair t-Test		0.1648	0.0434	0.0315	0.1419
Compared to Sample 1 Transport	414 55.8	385 52.9	382 53.9	385 55.3	431 61.0
Matched Pair t-Test		0.0001	0.0330	0.0608	0.2721
Compared to Sample 1					
LSMeans	0.9736	0.1692	0.1463	0.2254	0.7220
Comparison between Treatments					

Sphericity	0.0000
Sample	0.0001
Treat x Sample	0.0001
Treat	0.4016

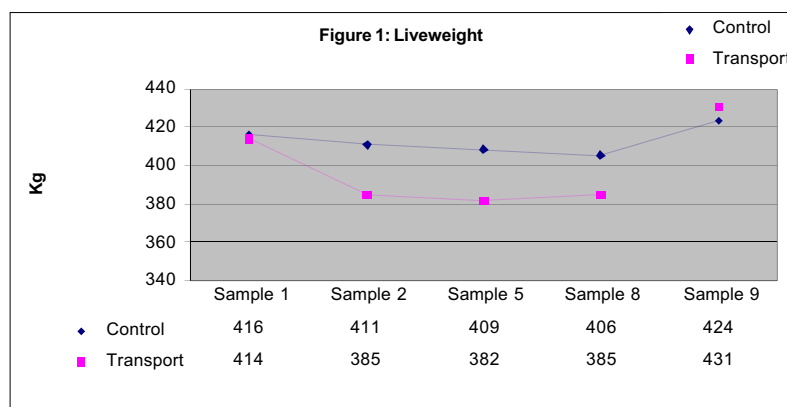


Table 5B: Animal performance and slaughter data for control and transported animals. The values are expressed mean \pm SD.

	Control	Transported
Final liveweight (kg)	639.11 \pm	670.00 \pm
	52.26	60.11
		P = 0.098
LWG/day to slaughter (kg/day)	1.175 \pm	1.546 \pm
	0.235	0.714
		P = 0.047
Days to slaughter	193.9 \pm	160.7 \pm
	20.82	43.16
Kill out %	56.7 \pm	60.5 \pm
	1.70	1.5
		P = 0.0001
Carcass weight (Kg)	371.77 \pm	417.87 \pm
	50.43	30.09
		P = 0.0032

Number of control animals in each conformation score category: Control R3 n=5; U3 n=10; U4-L n=1; U4-H n=1; R4-h n=1;

7.6 Physiological variables

Table 6: Normal biochemical ranges for cattle, from the Veterinary Laboratories Agency, and normal haematological ranges from Jain (1986) and Radostits and others (1994), Schalm (1961) and Kanenko (1989). Source reference (Knowles et al., 2000).

Biochemistry	Range	Haematology	Range
Haematocrit (%)	24–46	Albumin (g/litre)	27–39
Haemoglobin (g/dl)	8–15	ALP (U/litre)	90–170
Lymphocytes (10^9 /litre)	2.5–7.5	BHB (mmol/litre)	0–1.2
MCH (pg)	11–17	CK (U/litre)	0–200
MCHC (g/dl)	30–36	Cortisol *	
Mean Cell Volume (fl)	40–60	Creatinine (μ mol/litre)	44–165
Monocytes (10^9 /litre)	0–0.8	Iron (μ mol/litre)	21–41
Neutrophils (10^9 /litre)	0.6–4.0	Fibrinogen (g/litre)	2–5
Platelets (10^9 /litre)	100–800	Glucose (mmol/litre)	2.8–3.6
RBC (10^{12} /litre)	5–10	Haptoglobin (g/litre)	0–0.04
WBC (10^{12} /litre)	4–12	NEFA (μ mol/litre)	0–600
		Total protein (g/litre)	61–81
		Urea (mmol/litre)	3.4–7.3 *

No estimates available. MCH mean cell haemoglobin; MCHC mean cell haemoglobin concentration.

7.6.1 Protein

Protein measurement along with Albumin can indicate whether there has been an antibody response. (Total Protein–Albumin = Globulin) an increase in Gamma Globulins and a series of Acute Phase Proteins can result in an increase in the total Protein but this is usually somewhat offset by the reduction of Albumin in all Acute Phase situations. (Albumin is a “Reverse” Acute Phase Protein). Total Protein by itself can in no way diagnose liver damage or disease.

There was no significant difference in plasma protein concentrations prior to transport between control and transported animals (Table 7). Following transport, protein concentrations were significantly higher at sample 2 to 9 post-transport. Control animals had

significantly higher protein concentrations at sample 2 to 9. The Mann-Whitney U test comparison test showed that transported animals had significantly higher protein concentrations than controls at sample 2 (0 hours after arrival in France), sample 5, 7 and 8.

7.6.2 Albumin

There was no significant difference in blood albumin concentrations prior to transport (Table 8). There was a significant increase in albumin concentrations following the transport journey to France, and concentrations remained elevated at 12 and 24 hours after arrival and up to day 11 post transport and concentrations were significantly lower on day 40, compared with pre-transport baseline values. There was a significant treatment by sample interaction.

Plasma albumin concentrations were significantly higher in control animals and remained elevated up to day 11. By day 40 of the study, control animals had significantly lower albumin concentrations when compared to pre-transport values. Albumin concentrations returned to pre-transport control concentrations by sample 9 (day 40 of the study). Albumin measurements are used in the diagnosis and treatment of numerous diseases involving primarily the liver and kidney. Albumin has two major functions within the body. Albumin creates an osmotic gradient between the inside of blood vessels and the surrounding tissues. Without this, water migrates into the tissues and oedema develops. Normal concentrations of Albumin in the blood prevent this from happening. The only cause of increased albumin is dehydration; there is no naturally occurring hyperalbuminemia. Dehydration leads to hemoconcentration through reduction in fluid volume and consequently hyperprotinaemia.

7.6.3 Globulin

There was no significant difference in plasma globulin concentrations prior to transport (Table 9). There was a significant increase in globulin concentrations following the transport journey to France, and concentrations remained elevated at 12 (sample 3) and 24 hours (sample 4) after arrival in France and up to day 40 post transport. There was a significant treatment by sample interaction. Plasma

globulin concentrations were significantly higher in control animals and remained elevated up to day 40.

7.6.4 Red blood cell (RBC) numbers

There was no significant difference in red blood cell numbers prior to transport between control and transported animals (Table 10). RBC numbers were significantly increased at arrival in France (sample 2), 12 hours after arrival (sample 3), at arrival in Italy (sample 5) and significantly lower on day 40 (sample 9) of the study. Control animals had significantly higher concentrations at sample 3 and 4 (12 and 24 hours after arrival in France) and 6 (day 7 of the study) and lower number at sample 8 and 9 (day 11 and day 40).

7.6.5 Mean cell haemoglobin concentration (MCHC)

There was no significant difference in MCHC prior to transport between control and transported animals (Table 11). Transported animals had significantly higher MCHC at samples 5 (arrival in Italy), 6, 7, 8, and 9 post-transport. Control animals had significantly higher concentrations at sample 4, 8 and 9.

7.6.6 Haemoglobin

There was no significant difference in plasma haemoglobin concentrations prior to transport between control and transported animals (Table 12). There was a significant increase in haemoglobin concentrations following the transport journey to France, and concentrations remained elevated at arrival and 12 hours after arrival in France, and on day 5 of the study (arrival in Italy).

Plasma haemoglobin concentrations were significantly higher in control animals and remained elevated at sample 3, 4 and 6 (days 3, 4 and 7 of the study) and significantly lower at sample 8 and 9 (day 11 and 40 of the study).

7.6.7 Haematocrit

There was no significant difference in plasma globulin concentrations prior to transport (Table 13).

There was a significant increase in haematocrit percentage at sample 3 (day 3 of the study) and decrease at samples 6 (day 7) and sample 8 (day 11) in transported animals.

Control animals had significantly higher haematocrit percentage at sample 3 (day 3) and sample 6 (day 7) and significantly lower values at samples 8 and 9 (day 11 and 40 of the study).

7.6.8 White blood cell (WBC) numbers

There was no significant difference in white blood cell numbers prior to transport between control and transported animals (Table 14). Following transport, WBC numbers were significantly higher at sample 4 post-transport. Control animals had significantly lower WBC numbers at sample 2.

The Mann-Whitney U test comparison test showed that transported animals had significantly higher protein concentrations than controls at sample 2 (0 hours after arrival in France), sample 3, 4, 5, and 7.

7.6.9 Lymphocyte %

There was no significant difference in lymphocyte % prior to transport between control and transported animals (Table 15). Transported animals had significantly lower lymphocyte percentages at sample 2, (day 2; arrival in France time 0) and sample 4 (day 4 of the study)

7.6.10 Neutrophil %

There was no significant difference in neutrophil % prior to transport between control and transported animals (Table 16). There was a significant increase in neutrophil % at sample 6 and 7 post transport. Control animals had significantly lower neutrophil percentages at sample 2 and higher percentages at sample 8 (day 11 of the study).

7.6.11 Urea

There was no significant difference in urea concentrations prior to transport between control and transported animals (Table 17).

Plasma urea concentrations were significantly increased at sample 2 (arrival in France; time 0) and 3 (12 hours after arrival in France), and decreased at samples 4, 6, 8 and 9 post-transport. In control animals, plasma urea concentrations were significantly decreased from sample 2 to sample 8 of the study.

7.6.12 beta-hydroxy butyrate (β HB)

There was no significant difference in β HB concentrations prior to transport between control and transported animals (Table 18). Following transport, β HB concentrations were significantly increased at sample 2, 3, 4, 5, 6 and 8. Control animals had significantly higher β HB concentrations at samples 2, 3, 4, 5, 6 and 7. The Mann-Whitney U test comparison test showed that transported animals had significantly higher concentrations than controls at sample 2 and significantly lower concentrations than controls at sample 6 (day 7 of the study).

7.6.13 Creatine Kinase (CK)

It is also important to indicate that the CK activities of the transported animals while significantly higher than control values are still within the normal physiological range.

CK iso-enzymes are the most organ specific serum enzymes in clinical use. They catalyse the reversible phosphorylation of creatine to ATP to form creatine phosphate, the major storage form of high-energy phosphate required by muscle.

There was no significant difference in creatine kinase (CK) concentrations prior to transport between control and transported animals (Table 19). Following transport, CK concentrations were significantly increased at sample 2, 3, 4, 5, and significantly lower at sample 7, 8 and 9. Control animals had significantly lower CK concentrations at sample 6, 7, 8 and 9. The Mann-Whitney U test comparison test showed that transported animals had significantly higher concentrations than controls at sample 2, 3, 4 and 5.

7.6.14 Non-esterified fatty acid (NEFA)

There was no significant difference in plasma NEFA concentrations prior to transport between control and transported animals

(Table 20). Following transport, plasma NEFA concentrations were significantly higher at sample 2 to 8 and lower at sample 9 following transport. Control animals had significantly higher plasma haptoglobin concentrations at samples 2 to 8. The Mann-Whitney U test comparison test showed that transported animals had significantly lower plasma haptoglobin concentrations than controls at samples 2, 4, 5, 6, 7, 8.

7.6.15 Glucose

There was no significant difference in plasma glucose concentrations prior to transport between control and transported animals (Table 21). Following transport, plasma glucose concentrations were significantly lower at sample 5, 6, 7, 8, and 9. Control animals had significantly lower glucose concentrations at sample 5, 7, 8 and 9. The Mann-Whitney U test comparison test showed that transported animals had significantly lower plasma glucose concentrations than controls at sample 6 (day 7 of the study).

7.6.16 Concanavalin-A (Con-A) Interferon- γ (IFN- γ)

The mitogen (keyhole limpet haemocyanin KLH) – and antigen (Concanavalin-A (Con-A))-induced in vitro interferon- γ production was used as an indicator of cell-mediated immunity and is a useful and sensitive indicator of changes in immune function. As the technique does not necessarily require repeated differential centrifugation to isolate lymphocytes, it is also more practical from a methodological aspect than measurements of lymphocyte blastogenesis, with larger numbers of blood samples able to be handled at one time.

There was no significant difference in IFN- γ concentrations prior to transport between control and transported animals (Table 22). Following transport, IFN- γ concentrations were significantly lower at sample 6 and 7 (days 7 and 9 of the study). Control animals had significantly lower IFN- γ concentrations at sample 6 and 8. The Mann-Whitney U test comparison test showed that transported animals had significantly higher IFN- γ concentrations than controls at sample 9 (day 40 of the study).

7.6.17 Phytohaemagglutinin-A (PHA) Interferon- γ (IFN- γ)

There was no significant difference in IFN- γ concentrations prior to transport between control and transported animals (Table 23). Following transport, IFN- γ concentrations were significantly lower at sample 4 and 7 (days 4 and 9 of the study). Control animals had significantly lower IFN- γ concentrations at sample 6 (day 7 of the study). The Mann-Whitney U test comparison test showed that transported animals had significantly lower IFN- γ concentrations than controls at sample 3 (12 hours after arrival in France).

7.6.18 Haptoglobin

There was no significant difference in plasma haptoglobin concentrations prior to transport between control and transported animals (Table 24). Following transport, plasma haptoglobin concentrations were significantly higher at sample 2 to 9 following transport. Control animals had significantly higher plasma haptoglobin concentrations at samples 3, 4, 5, 6, 7, and 8. The Mann-Whitney U test comparison test showed that transported animals had significantly lower plasma haptoglobin concentrations than controls at sample 9 (day 40 of the study).

7.6.19 Fibrinogen

There was no significant difference in plasma fibrinogen concentrations prior to transport between control and transported animals (Table 25). There was a significant increase in fibrinogen concentrations following the transport journey to France, and concentrations remained elevated at 12 and 24 hours after arrival and up to day 40 of the study. There was a significant treatment by sample interaction. Plasma fibrinogen concentrations were significantly higher in control animals and remained elevated up to day 40.

Inflammation resulting from stress can cause the release of acute phase proteins (APP) such as haptoglobin and fibrinogen, and APP in cattle have been associated with immunosuppression and much higher concentrations have been reported in inflammatory conditions (Earley et al., 2002).

7.6.20 Serum antibody titers for respiratory viruses.

There was no significant difference in serum antibody concentrations for infective bovine rhinotracheitis virus (IBR) prior to or after transport between control and transported animals (Table 26).

There was no significant difference in serum antibody concentrations for the respiratory syncytial virus (RSV) prior to transport or at samples 1, 4, 5, 7 for control and transported animals (Table 27).

Control animals had significantly higher RSV antibody titers at day 40 (sample 9) compared with pre-transport baseline values. Transported animals had significantly higher RSV titers at sample bleed 8 and 9 (days 11 and 40 respectively).

There was no significant difference in serum antibody concentrations for the para-influenza-3 (PI-3) virus at samples 1, 4, 5, 7 for control and transported animals (Table 28). Control and transported animals had significantly higher PI-3 titers at sample bleed 9 (day 40).

8. CONCLUSION.

The physiological, immunological and haematological effects on bulls of transporting them from Ireland to Italy, including a 24-hour rest period at a staging post in France, with access to water on the transporter, were studied in 26 transported bulls and 22 control bulls. The mean liveweight of the transported group decreased by 7.0% by the time of arrival at the staging post in Fougères, France. On day 10 of the study, 5 days after arrival in Italy, an outbreak of pneumonia occurred in the animals in the feedlot in Italy. One animal died and all animals were vaccinated (Rispoval and Immuresp) against respiratory syncytial virus (RSV) and *Pasteurella* (Presponse). In Italy, animals showing signs of pneumonia were injected with the antibiotics Amoxil (long acting) and Micotil. Two bulls died on the control farm in Ireland following an outbreak of respiratory disease which occurred after day 11 of the study.

The study concluded that transport had no adverse effect on animal welfare based on the physiological, immunological and behavioural measurements made. The physiological measurements indicated that the journey periods were not excessively physically demanding, and bulls spent 64% of the journey time on the ferry resting. Physiological measurements made after the journeys indicated that 24 hours in lairage, with hay and water freely available, allowed the animals to recover substantially, although not completely, irrespective of the journey time.

Based on the immunological and physiological measurements made and the behavioural observations the transport journey under the present conditions is not unacceptable from the viewpoint of animal welfare.



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Table 4: Rectal body temperature (°C) in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Mean ± SD with P values.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Control	39.1	39.0	38.9	39.1	38.9	39.0	39.3	39.6	39.2
	0.55	0.46	0.44	0.46	0.38	0.36	0.68	0.67	0.37
Matched Pair t-Test Compared to Sample 1		0.5580	0.1526	0.8835	0.1313	0.6996	0.3815	0.0088	0.6097
Transport	39.3	39.3	39.0	39.4	39.2	39.5	39.9	39.1	39.2
	0.60	0.59	0.68	0.59	0.44	0.98	0.81	0.74	0.34
Matched Pair t-Test Compared to Sample 1		0.8585	0.1333	0.3450	0.5434	0.0034	0.3346	0.4536	
LSMeans	0.5254	0.0962	0.3004	0.0672	0.0290	0.1195	0.0107	0.0031	0.5177
Comparison between Treatments									

REPEATED MEASURES ANALYSIS

Sphericity	0.0016
Sample	0.0007
Transport x Sample	0.0007
Transport	0.0494

Table 7 : Protein concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Median with minimum and Maximum values with P values.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Control	73.4	77.7	78.8	77.9	77.1	78.7	80.8	80.2	83.7
	66.4-82.3	71.5-84.9	72.8-88.8	74.2-90.0	72.2-88.4	70.1-92.7	71.7-92.0	73.2-91.2	68.6-102.0
Wilcoxon	0.0059	0.0015	0.0015	0.0132	0.0017	0.0011	0.0001	0.0001	
Compared to Sample 1									
Transport	75.2	85.4	81.1	79.5	81.9	79.5	84.3	86.6	81.4
	42.2-81.0	69.8-93.1	70.1-91.5	68.7-92.2	73.6-96.1	73.7-93.3	71.3-102.9	76.2-95.3	73.7-112.9
Wilcoxon	0.0001	0.0001	0.0012	0.0001	0.0001	0.0001	0.0001	0.0001	
Compared to Sample 1	ns	T > C	ns	ns	T > C	ns	T > C	T > C	ns
Mann-Whitney Comparison between Treatments.									
REPEATED MEASURES ANALYSIS									
Sphericity	0.0014								
Sample	0.0001								
Treat x Sample	0.0001								
Treat	0.0254								

Table 8: Plasma albumin concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Mean \pm SD with P values.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Control	37.16	39.01	39.68	39.29	39.63	39.98	39.48	38.92	36.56
Matched Pair t-Test	1.111	2.011	1.631	1.373	1.470	2.087	1.727	1.818	1.420
Compared to Sample 1		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.2271
Transport	37.48	41.17	40.29	39.06	40.27	39.91	38.57	39.21	36.48
Matched Pair t-Test	1.462	1.772	1.512	1.620	1.732	1.509	1.332	1.828	1.879
Compared to Sample 1		0.0001	0.0001	0.0001	0.0001	0.0001	0.0082	0.0001	0.0050
LSMeans	0.2796	0.0001	0.1482	0.8131	0.1464	0.7512	0.1935	0.3153	0.8668
Comparison between Treatments									

REPEATED MEASURES ANALYSIS

Sphericity	0.0049
Sample	0.0001
Treat x Sample	0.0009
Treat	0.0235

Table 9: Plasma globulin concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Mean \pm SD with P values.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Control	36.8	38.8	39.3	39.7	38.1	39.1	40.9	41.9	47.6
Matched Pair t-Test	4.33	3.75	4.34	4.49	4.03	4.35	5.03	4.50	8.41
Compared to Sample 1		0.0005	0.0001	0.0001	0.0266	0.0089	0.0001	0.0001	0.0001
Transport	38.1	43.3	41.2	40.8	41.8	41.4	45.6	47.5	46.9
Matched Pair t-Test	3.84	4.68	3.99	4.64	4.42	4.63	5.68	5.07	9.64
Compared to Sample 1		0.0001	0.0001	0.0004	0.0002	0.0021	0.0001	0.0001	0.0003
LSMeans	0.6258	0.0067	0.3281	0.7430	0.0176	0.1606	0.0075	0.0002	0.8099
Comparison between Treatments									
REPEATED MEASURES ANALYSIS									
Sphericity	0.0001								
Sample	0.0001								
Treat x Sample	0.0001								
Treat	0.0603								

Table 10: Red blood cell (RBC) numbers in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Mean \pm SD with P values.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Control	11.1 0.78	11.8 1.09 0.0163	12.2 0.96 0.0001	11.8 0.88 0.0066	11.4 0.85 0.2864	11.7 1.00 0.0239	11.3 0.93 0.5278	10.5 1.14 0.0296	10.3 0.83 0.0019
Matched Pair t-Test Compared to Sample 1									
Transport	10.6 0.99	11.8 1.22 0.0001	11.3 0.84 0.0065	10.9 0.88 0.1944	11.2 1.16 0.0276	10.5 1.27 0.3996	10.5 1.21 0.6905	10.3 1.16 0.1648	10.0 1.10 0.0164
Matched Pair t-Test Compared to Sample 1									
LSMeans	0.1843	0.8449	0.0049	0.0027	0.8494	0.0027	0.0244	0.4112	0.3140
Comparison between Treatments									
REPEATED MEASURES ANALYSIS									
Sphericity	0.0002								
Sample	0.0001								
Treat x Sample	0.0001								
Treat	0.0455								

Table 11: Mean cell haemoglobin concentration (MCHC) in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Mean \pm SD with P values.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Control	34.4	34.4	34.4	34.9	34.6	34.3	34.4	34.5	35.2
Matched Pair t-Test	0.61	0.74	0.50	0.56	0.86	0.86	0.81	0.66	0.74
Compared to Sample 1		0.6893	0.7183	0.0009	0.2291	0.9553	0.8692	0.1443	0.0002
Transport	34.7	35.0	34.4	34.9	35.3	35.7	36.2	35.8	38.1
Matched Pair t-Test	0.82	2.09	0.66	0.63	0.79	0.78	0.73	0.94	0.86
Compared to Sample 1		0.4272	0.2110	0.0191	0.0001	0.0001	0.0002	0.0001	
LSMeans	0.2162	0.2509	0.7418	0.8750	0.0077	0.0001	0.0001	0.0001	0.0001
Comparison between Transportments									

REPEATED MEASURES ANALYSIS

Sphericity 0.0011
 Sample 0.0001
 Treat x Sample 0.0001
 Treat 0.0001

Table 12: Haemoglobin concentration (Hb) in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Mean \pm SD with P values.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Control	13.2	13.7	14.2	14.0	13.4	13.7	13.3	12.4	12.2
Matched Pair t-Test	1.10	1.21	1.07	0.92	0.94	1.34	1.21	1.19	1.08
Compared to Sample 1		0.0974	0.0035	0.0150	0.4112	0.0502	0.7699	0.0032	0.0022
Transport	12.6	13.7	13.0	12.7	13.1	12.4	12.7	12.2	12.4
Matched Pair t-Test	0.86	1.33	1.09	1.09	1.22	1.40	1.37	1.44	1.05
Compared to Sample 1		0.0011	0.0461	0.6031	0.0203	0.6288	0.6642	0.2479	0.3501
LSMeans	0.0775	0.7349	0.0011	0.0003	0.6703	0.0051	0.0901	0.5860	0.6776
Comparison between Treatments									
REPEATED MEASURES ANALYSIS									
Sphericity	0.0003								
Sample	0.0001								
Treat x Sample	0.0001								
Treat	0.0568								

Table 13: Haematocrit (HCT) percentage in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Mean \pm SD with P values.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Control	38.5	39.9	41.4	40.1	38.7	39.8	38.6	35.9	34.7
	3.27	3.48	3.14	2.74	2.85	3.50	3.63	3.44	3.15
Matched Pair t-Test		0.1226	0.0026	0.0721	0.6538	0.0424	0.7853	0.0024	0.0004
Compared to Sample 1									
Transport	36.2	39.1	37.7	36.3	37.2	36.0	35.0	34.2	31.7
	2.70	4.08	3.15	3.12	3.62	7.53	3.68	3.85	4.84
Matched Pair t-Test	0.0007	0.0339	0.8844	0.1871	0.9259	0.1592	0.0171	0.0004	
Compared to Sample 1									
LSMeans	0.0464	0.4119	0.0012	0.0003	0.3228	0.0844	0.0021	0.1111	0.0279
Comparison between Treatments									
REPEATED MEASURES ANALYSIS									
Sphericity	0.0037								
Sample	0.0001								
Treat x Sample	0.0001								
Treat	0.0058								

Table 14 : White blood cell (WBC) numbers in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Median with minimum and Maximum values with P values.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Control	10.3 7.8-18.1	9.1 5.9-14.4	8.8 6.2-14.8	9.9 6.9-17.5	9.1 6.4-21.7	9.8 7.5-16.3	8.8 5.4-19.6	10.5 5.7-24.3	8.8 6.3-14.1
Wilcoxon		0.0391	0.0894	0.2966	0.1046	0.4428	0.2628	0.9699	0.0930
Compared to Sample 1									
Treat	10.5 7.0-17.8	12.1 6.1-33.1	11.5 6.8-28.9	12.6 8.3-19.0	9.7 5.8-21.4	10.3 6.5-30.7	13.1 8.0-53.6	9.7 6.8-14.5	9.9 7.3-15.3
Wilcoxon		0.2305	0.1310	0.0153	0.1586	0.9271	0.0714	0.0934	0.1222
Compared to Sample 1	ns	T > C	T > C	T > C	T > C	ns	T > C	ns	ns
Mann-Whitney Comparison between Treatments.									

REPEATED MEASURES ANALYSIS

Sphericity 0.0011
 Sample 0.0001
 Treat x Sample 0.0018
 Treat 0.0111

Table 15: Lymphocyte % in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Mean ± SD with P values.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Control	56	60	53	53	54	55	50	46	59
	10.5	10.6	10.8	12.3	13.9	10.0	15.8	18.4	12.3
Matched Pair t-Test		0.0436	0.1547	0.3953	0.5600	0.7975	0.0790	0.0052	0.2373
Compared to Sample 1									
Transport	57	48	53	49	56	52	54	58	58
	8.7	15.4	15.8	17.1	11.5	14.1	13.2	8.1	8.3
Matched Pair t-Test		0.0113	0.2016	0.0391	0.8819	0.2732	0.3989	0.5235	0.4881
Compared to Sample 1									
LSMeans	0.5420	0.0134	0.8591	0.2519	0.9423	0.4311	0.6104	0.0198	0.7292
Comparison between Treatments									

REPEATED MEASURES ANALYSIS

Sphericity 0.11199
 Sample 0.2502
 Treat x Sample 0.0629
 Treat 0.7460

Table 16: Neutrophil % in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Mean \pm SD with P values.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Control	37.86	30.81	39.00	39.35	37.38	36.38	43.29	49.38	34.26
Matched Pair t-Test	10.036	11.093	10.621	12.504	14.665	11.187	16.605	19.730	12.364
Compared to Sample 1		0.0025	0.6103	0.6932	0.8821	0.5509	0.1316	0.0045	0.1896
Transport	34.62	40.38	33.12	38.73	39.69	43.65	42.92	36.92	34.12
Matched Pair t-Test	9.667	13.488	14.292	14.766	11.821	9.814	12.630	8.266	8.638
Compared to Sample 1		0.0946	0.5730	0.2663	0.0653	0.0036	0.0152	0.3701	0.7837
LSMeans	0.2360	0.0197	0.3151	0.8661	0.3298	0.0128	0.7828	0.0281	0.8938
Comparison between Treatments									
REPEATED MEASURES ANALYSIS									
Sphericity	0.0403								
Sample	0.0148								
Treat x Sample	0.0293								
Treat	0.7365								

Table 17: plasma urea concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Mean \pm SD with P values.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Control	5.1	4.3	4.2	4.0	4.1	4.3	4.3	3.9	4.4
Matched Pair t-Test	1.17	1.26	0.75	0.89	0.68	0.72	0.67	0.73	1.36
Compared to Sample 1	0.0032	0.0005	0.0001	0.0004	0.0004	0.0032	0.0028	0.0001	0.1347
Transport	5.5	7.6	6.1	4.8	5.4	4.2	4.9	4.9	3.1
Matched Pair t-Test	1.47	1.33	1.34	1.09	0.76	0.90	1.18	1.49	0.69
Compared to Sample 1	0.0001	0.0327	0.0030	0.0006	0.6110	0.0006	0.0823	0.0779	0.0001
LSMeans	0.2409	0.0001	0.0001	0.0139	0.0001	0.4400	0.0897	0.0094	0.0002
Comparison between Treatments									

REPEATED MEASURES ANALYSIS

Sphericity	0.0008
Sample	0.0001
Treat x Sample	0.0001
Treat	0.0001

Table 18 : Plasma beta-hydroxybutyrate (BHB) concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Median with minimum and Maximum values with P values.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Control	0.19 0.08-0.44	0.26 0.18-0.49	0.33 0.20-0.55	0.32 0.16-0.55	0.28 0.12-0.39	0.29 0.23-0.40	0.28 0.11-0.57	0.22 0.05-0.61	0.26 0.06-0.40
Wilcoxon		0.0006	0.0001	0.0001	0.0010	0.0001	0.0162	0.5122	0.0332
Compared to Sample 1									
Transport	0.20 0.08-0.42	0.33 0.09-0.53	0.28 0.11-0.49	0.29 0.12-0.50	0.29 0.02-0.63	0.24 0.03-0.59	0.21 0.04-0.69	0.25 0.13-0.82	0.22 0.10-0.38
Wilcoxon		0.0013	0.0069	0.0009	0.0017	0.1903	0.6012	0.0112	0.1150
Compared to Sample 1	ns	T > C	ns	ns	ns	T > C	ns	ns	ns
Mann-Whitney Comparison between Treatments.									

REPEATED MEASURES ANALYSIS

Sphericity 0.0081
 Sample 0.0001
 Treat x Sample 0.0001
 Treat 0.1755

Table 19 : Creatine Kinase (CK) concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (clay 0). Values are expressed as Median with minimum and Maximum values with P values.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Control	203 124-1550	289 134-492	227 115-501	247 148-407	229 145-2094	177 160-728	138 92-920	139 76-370	142 84-307
Wilcoxon		0.1344	0.3585	0.2130	0.8999	0.0469	0.0111	0.0026	0.0026
Compared to Sample 1									
Transport	185 100-440	448 143-2079	499 160-1527	343 121-915	298 117-663	152 75-449	123 72-294	124 62-379	130 88-231
Wilcoxon		0.0001	0.0001	0.0001	0.0017	0.0728	0.0023	0.0033	0.0002
Compared to Sample 1	ns	T > C	T > C	T > C	T > C	ns	ns	ns	ns
Mann-Whitney Comparison between Treatments.									
REPEATED MEASURES ANALYSIS									
Sphericity	0.0003								
Sample	0.0001								
Treat x Sample	0.0006								
Treat	0.4022								

Table 20 : Plasma non-esterified fatty acid (NEFA) concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Median with minimum and Maximum values with P values.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Control	0.15 0.06-0.31	0.32 0.07-0.92	0.51 0.21-1.35	0.33 0.16-0.74	0.34 0.12-0.83	0.30 0.18-0.82	0.26 0.15-1.29	0.27 0.13-0.92	0.13 0.06-0.52
Wilcoxon Compared to Sample 1		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0002	1.0000
Transport	0.16 0.09-0.35	0.74 0.31-1.32	0.58 0.32-1.32	0.46 0.19-1.35	0.62 0.34-2.24	0.53 0.06-1.31	0.57 0.30-1.05	0.51 0.24-1.32	0.12 0.06-0.27
Wilcoxon Compared to Sample 1		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0271
Mann-Whitney Comparison between Treatments.	ns	T > C	ns	T > C	T > C	T > C	T > C	T > C	ns

REPEATED MEASURES ANALYSIS

Sphericity 0.0216
 Sample 0.0001
 Treat x Sample 0.0001
 Treat 0.0001

Table 21: Plasma glucose concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Median with minimum and Maximum values with P values.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Control	4.8 4.1-6.4	4.9 4.2-6.0	4.8 4.4-5.3	4.9 4.1-5.7	4.5 4.1-5.9	4.7 3.7-5.3	4.4 3.7-5.1	4.4 4.0-7.3	4.3 3.9-7.1
Wilcoxon		0.9698	0.5610	0.7813	0.0318	0.0708	0.0011	0.0035	0.0002
Compared to Sample 1									
Transport	4.8 3.9-6.3	5.0 4.3-6.3	4.7 3.7-5.6	4.6 3.7-5.6	4.5 3.8-5.7	4.2 3.7-4.9	4.4 3.0-5.1	4.3 3.7-4.8	4.5 3.4-6.0
Wilcoxon		0.2399	0.2623	0.0809	0.0283	0.0001	0.0002	0.0001	0.0226
Compared to Sample 1	ns	ns	ns	ns	ns	C > T	ns	ns	ns
Mann-Whitney Comparison between Treatments.									

REPEATED MEASURES ANALYSIS

Sphericity	0.0008
Sample	0.0001
Treat x Sample	0.0055
Treat	0.1825

Table 22: Concanavalin-A (Con-A) induced interferon- γ (IFN- γ) concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Median with minimum and Maximum values with P values.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Control	0.147 -0.055-0.415	0.157 0.025-0.425	0.151 -0.004-0.648	0.108 -0.013-0.358	0.124 -0.015-0.363	0.059 -0.051-0.387	0.079 -0.003-0.519	0.045 0.016-0.382	0.109 0.003-0.331
Wilcoxon	0.4656	0.3328	0.4504	0.3924	0.0221	0.2572	0.0235	0.8074	
Compared to Sample 1									
Transport	0.116 -0.006-1.012	0.234 -0.076-1.419	0.161 -0.048-0.558	0.131 -0.008-0.380	0.195 -0.001-0.842	0.053 -0.010-0.823	0.063 -0.003-0.487	0.099 0.002-0.444	0.224 0.017-0.858
Wilcoxon	0.0972	0.6342	0.7007	0.4078	0.0395	0.0481	0.5217	0.0797	
Compared to Sample 1	ns	ns	ns	ns	ns	ns	ns	T > C	
Mann-Whitney Comparison between Treatments.									

REPEATED MEASURES ANALYSIS

Sphericity 0.0192
 Sample 0.0053
 Treat x Sample 0.5719
 Treat 0.7343

Table 23: Phytohaemagglutinin-A (PHA) induced interferon- γ (IFN- γ) concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Median with minimum and Maximum values with P values.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Control	0.445 0.146-1.336	0.659 0.147-1.985	0.737 0.169-1.843	0.376 0.037-1.583	0.258 -0.008-1.655	0.263 0.043-0.960	0.380 0.030-1.367	0.340 0.031-0.922	0.491 0.081-1.099
Wilcoxon	0.3391	0.1445	0.1445	0.1665	0.0527	0.0325	0.1445	0.0741	0.7046
Compared to Sample 1									
Transport	0.466 0.073-2.003	0.618 0.043-2.124	0.431 -0.063-1.515	0.295 0.052-2.714	0.519 0.024-1.500	0.369 0.020-1.640	0.345 0.017-2.000	0.548 0-1.277	0.411 0.079-1.205
Wilcoxon	0.9126	0.3186	0.3186	0.0345	0.9423	0.0805	0.0471	0.8183	0.1156
Compared to Sample 1	ns	ns	C > T	ns	ns	ns	ns	ns	ns
Mann-Whitney Comparison between Treatments.									

REPEATED MEASURES ANALYSIS

Sphericity	0.0057
Sample	0.0028
Treat x Sample	0.0254
Treat	0.5675

Table 24: Plasma haptoglobin concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Median with minimum and Maximum values with P values.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Control	0.27 0.18-1.35	0.29 0.21-0.79	0.34 0.24-0.75	0.37 0.22-1.03	0.55 0.25-3.60	0.95 0.23-3.71	1.20 0.25-5.43	1.69 0.25-5.67	0.24 0.16-2.66
Wilcoxon	0.2841	0.0039	0.0037	0.0003	0.0001	0.0001	0.0001	0.0001	0.7860
Compared to Sample 1									
Transport	0.24 0.15-0.55	0.33 0.19-0.91	0.29 0.20-2.19	0.33 0.07-2.66	0.59 0.10-3.27	1.31 0.13-5.20	2.42 0.12-4.85	2.13 0.14-4.45	0.13 0.09-2.82
Wilcoxon	0.0009	0.0075	0.0026	0.0115	0.0001	0.0001	0.0001	0.0001	0.0001
Compared to Sample 1	ns	ns	ns	ns	ns	ns	ns	ns	C > T
Mann-Whitney Comparison between Treatments.									

REPEATED MEASURES ANALYSIS

Sphericity 0.0009
 Sample 0.0001
 Treat x Sample 0.0500
 Treat 0.6410

Table 25: Plasma fibrinogen concentrations in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Median with minimum and Maximum values with P values.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Control	574	699	643	642	897	953	1129	1242	841
Matched Pair t-Test	90.9	118.1	59.3	110.7	212.4	284.6	388.4	425.9	260.2
Compared to Sample 1		0.0001	0.0015	0.0242	0.0001	0.0001	0.0001	0.0001	0.0004
Treat	541	693	641	698	875	1023	1447	1462	651
Matched Pair t-Test	99.7	136.6	145.5	202.4	261.0	360.7	412.8	406.7	184.4
Compared to Sample 1		0.0001	0.0001	0.0009	0.0001	0.0001	0.0001	0.0001	0.0144
LSMeans	0.2480	0.9797	0.9979	0.2436	0.7789	0.3025	0.0003	0.0063	0.0071
Comparison between Treatments									

REPEATED MEASURES ANALYSIS

Sphericity	0.0017
Sample	0.0001
Treat x Sample	0.007
Treat	0.0846

Table 26 : Serum antibody concentrations for Infective bovine rhino-tracheitis virus (IBR) in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Median with minimum and Maximum values with P values.

	Sample 1	Sample 4	Sample 5	Sample 7	Sample 8	Sample 9
Control	0.022	0.020	0.026	0.025	0.019	0.031
	0.003-0.822	0.002-0.852	-0.003-0.865	-0.002-1.038	-0.002-0.951	-0.005-1.366
Wilcoxon- Versus Sample 1	0.6870	1.0000	0.8998	0.9498	0.4966	
Transport	0.024	0.015	0.021	0.019	0.012	0.125
	-0.002-0.792	-0.003-2.513	-0.002-2.598	-0.003-2.503	-0.003-2.457	0-2.034
Wilcoxon- Versus Sample 1	0.9714	0.8261	0.8907	0.6209	0.3137	0.0801
LS Means		0.6761	0.8378	0.8078	0.3539	0.3181

REPEATED MEASURES ANALYSIS

Sphericity 0.0009
Sample 0.0001
Bleed x Treat 0.1984
Treat 0.9685

Table 27: Serum antibody concentrations for respiratory syncytial (RSV) virus in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Median with minimum and Maximum values with P values.

	Sample 1	Sample 4	Sample 5	Sample 7	Sample 8	Sample 9
Control	0.002 -0.001-0.020	0.002 -0.008-0.063	0.002 -0.004-0.059	0.002 -0.023-0.034	0.002 -0.019-0.125	0.269 0-0.673
Wilcoxon- Versus Sample 1		0.3968	0.8293	1.0000	0.6851	0.0001
Transport	0.007 -0.024-0.286	0.008 -0.012-0.172	0.003 -0.012-0.179	0.006 -0.005-0.845	0.062 0-1.281	0.527 0-1.397
Wilcoxon- Versus Sample 1		0.6802	0.1752	0.4692	0.0079	0.0001
LS Means	0.0339	0.0519	0.6147	0.2451	0.0001	0.8107

REPEATED MEASURES ANALYSIS

Sphericity 0.0000
Sample 0.0248
Bleed x Treat 0.1984
Treat 0.9685

Table 28: Serum antibody concentrations for para-influenza-3 (PI-3) virus in control and transported animals (samples 1 to 9). Pre-transport measurements were taken at -24 hours (day 0). Values are expressed as Median with minimum and Maximum values with P values.

	Sample 1	Sample 4	Sample 5	Sample 7	Sample 8	Sample 9
Control	0.066 0.003-0.872	0.046 0.002-0.852	0.023 -0.003-0.865	0.034 -0.002-1.038	0.089 -0.002-0.951	0.963 -0.005-1.366
Wilcoxon- Versus Sample 1		0.5628	0.5628	0.5543	0.6060	0.0001
Transport	0.038 -0.002-0.792	0.018 -0.003-2.513	0.014 -0.002-2.598	0.015 -0.003-2.503	0.035 -0.003-2.457	1.036 0-2.034
Wilcoxon- Versus Sample 1		0.4100	0.3363	0.2841	0.9850	0.0001
LS Means	0.5883	0.2034	0.2134	0.1754	0.3615	0.2810

REPEATED MEASURES ANALYSIS

Sphericity 0.0000
Sample 0.0001
Bleed x Treat 0.6330
Treat 0.2720