

Health of non-ambulatory, non-injured pigs at processing

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Abstract

Loss of pigs during or after transport is a welfare concern, but also an economic concern for producers. Transport losses include animals that are dead on arrival (DOA) at the plant, pigs that are injured, and pigs which are not obviously injured but unwilling or unable to walk (non-ambulatory, non-injured or NANI). The objective of this research was to assess the health of non-ambulatory, non-injured (NANI) pigs relative to control pigs at the processing plant by looking at a range of measures, including complete blood chemistry, anatomy, and pathology to determine potential factors associated with pigs going down. Data were collected from NANI and control pigs at five plants in the midwest USA. Feet and legs and internal organs were inspected and the severity of the pathology scored. Alveolar macrophages were collected and counted. Blood was collected for analysis of hematology, blood chemistry and cortisol concentrations. Titers to common porcine respiratory viruses were measured in pigs from one plant. Hoof and pad problems did not differ overall between control and NANI pigs, however the percentage of severe foot problems was greater ($P < 0.05$) in NANI compared with control pigs at plants A and E. The percentage of total ulcers, rhinitis, and empty stomachs differed ($P < 0.05$) between control and NANI pigs at individual plants, but not overall. Blood hematology and chemistry differed ($P < 0.05$) between NANI and control pigs. Cortisol concentrations did not differ between NANI and control pigs. Titers to swine influenza virus (SIV) H1N1 and H3N2 and porcine circovirus (PCV) were lower ($P < 0.01$) among NANI compared with control pigs. However, more ($P < 0.01$) NANI pigs were positive for SIV H1N1 and H3N2 compared with control pigs. Blood hematology, chemistry, and pathology indicate a large difference between NANI and controls pigs. No single health problem was higher among NANI pigs compared to plant-matched control pigs. Rather, several problems appear to contribute to pigs becoming NANI which may differ from one plant to another.

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1. Introduction

Losses of pigs during or after transport are both welfare and economic concerns. Transport losses include animals that are dead on arrival at the packing plant, pigs that have difficulty in walking during unloading,

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Table 1

Feet and hoof problem scores, % of the group, and gender of non-ambulatory, non-injured (NANI) and control pigs from four different processing plants in the United States

Measure	Plant A		Plant B		Plant D		Plant E		Plant averages	
	NANI	Control	NANI	Control	NANI	Control	NANI	Control	NANI	Control
<i>N</i>	39	39	36	36	60	60	62	57	197	192
Hoof damage/injury										
No. score 1	24.0	33.0	21.0	10.0	21.0	25.0	24.0	21.0	22.5	22.3
No. score 2	3.0	0.0	2.0	11.0 ^a	10.0	10.0	9.0	3.0	6.0	6.0
Total score	27.0	33.0	23.0	21.0	31.0	35.0	33.0	24.0	28.5	28.3
% hoof problems	69.2	84.6 ^a	63.9	58.3	51.7	58.3	53.2	42.1	59.5	60.8
Pad damage/injury										
No. score 1	11.0	4.0	20.0	13.0	9.0	14.0	17.0	19.0	14.3	12.5
No. score 2	3.0	0.0	8.0	17.0	25.0	20.0	28.0	14.0	16.0	12.8
Total score	14.0	4.0	28.0	30.0	34.0	34.0	45.0	33.0	30.3	25.3
% Pad problems	35.9	10.3 ^a	77.8	83.3	56.7	56.7	72.6	57.9	60.7	52.0
Total feet and leg injuries, %	105.1	94.9	141.7	141.7	108.3	115.0	125.8	100.0 ^a	108.5	105.0
Severe foot problems, %	15.4	0.0 ^a	27.8	77.8 ^a	58.3	50.0	59.7	29.8 ^a	37.5	38.5
Barrows, %			47.2	33.3	65.0	41.7 ^a	37.1	47.4	49.8	40.8
Gilts, %			52.8	66.7	30.0	51.7 ^a	62.9	50.9	48.6	56.4

^a Measures for NANI pigs for each plant and overall differ significantly from controls at $P < 0.05$.

commonly referred to as ‘downers’, ‘fatigued’, ‘subjects’, ‘suspects’, ‘slows’, or ‘NANI (non-ambulatory, non-injured)’ pigs (Ellis et al., 2003).

Currently, it is not known why some pigs die or go down during transport (Ellis et al., 2003). Speculation into the factors that affect the percentage of dead and NANI pigs during transport include genetics (Ellis et al., 2003), handling of pigs prior to and after transport (Peeters et al., 2004), the stress caused by mixing with conspecifics, exposure to a novel environment and health problems (Clark, 1979). Non-ambulatory, non-injured pigs can

exhibit symptoms that are characteristic of an acute stress response, including open-mouth breathing, skin discoloration, and muscle tremors (Ellis et al., 2003).

Severe and diffuse pulmonary congestion and edema were found in 70% of necropsied market weight pigs dead on arrival after transport (Clark, 1979). However, health related causes of NANI pigs are largely unknown. Hematological and blood chemistry profiles in slaughter weight pigs have been correlated to pathological–anatomical lesions, inflammatory processes and abscesses (Odink et al., 1990; Smeets et al., 1990; Elbers et al.,

Table 2

Internal measure scores of non-ambulatory, non-injured (NANI) and control pigs from four different processing plants in the United States

Measure	Plant B		Plant C		Plant D		Plant E		Plant averages	
	NANI	Control	NANI	Control	NANI	Control	NANI	Control	NANI	Control
Internal measures										
<i>N</i>	29	29	15	15	32	31	48	50	124	125
% Stomachs empty	58.6	48.3	20.0	6.7 ^a	15.6	6.5 ^a	4.2	24.0 ^a	24.6	21.3
Ulcers, score 1	6.0	3.0	1.0	1.0	9.0	1.0 ^a	1.0	6.0	4.3	2.8
Ulcers, score 2	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.3	0.0
Ulcers, total score	6.0	3.0	1.0	1.0	10.0	1.0 ^a	1.0	6.0	4.5	2.8
Total ulcers, %	20.7	10.3 ^a	6.7	6.7	31.3	3.2 ^a	2.1	12.0 ^a	15.2	8.1
Rhinitis, score 1			4.0	5.0	4.0	8.0			4.0	6.5
Rhinitis, score 2			4.0	1.0	5.0	6.0			4.5	3.5
Rhinitis, score 3			2.0	2.0	2.0	5.0			2.0	3.5
Rhinitis, score 4			0.0	2.0	2.0	1.0			1.0	1.5
Rhinitis, total score			10.0	10.0	13.0	20.0			11.5	15.0
Average rhinitis score			66.7	66.7	40.6	64.5 ^a			53.6	65.6
Total rhinitis, %			1.5	1.3	1.3	1.1			1.4	1.2
Liver, total score	9.0	0.0 ^a	1.0	0.0	8.0	7.0	8.0	5.0	6.5	3.0
Total liver, %	31.0	0.0 ^a	6.7	0.0 ^a	25.0	22.6	16.7	10.0	19.8	8.1
% Lung consolidation	8.4	2.4	1.6	3.9	5.9	8.3	3.7	2.2	4.9	4.2

^a Measures for NANI pigs for each plant and overall differ significantly from controls at $P < 0.05$.

Table 3

Macrophage sub-population in bronchoalveolar fluid of non-ambulatory, non-injured (NANI) and control pigs from four different processing plants in the United States

Measure	Plant B		SE	Plant C		SE	Plant D		SE	Plant E		SE	P-value Trt* Plant
	NANI	Control		NANI	Control		NANI	Control		NANI	Control		
N	36	36		8	7		8	9		58	46		
Subpop.1, %	71.3	72.4	2.40	28.8	24.1	4.01	30.9	37.8	3.68	50.8	59.3	6.70	0.440
Subpop.2, %	22.3	22.6	2.03	38.5	29.9	3.40	35.1	27.7	3.11	27.5	27.3	5.67	0.297
Subpop.5, %	6.4	5.0	1.50	32.7	46.0 ^a	2.51	34.0	34.6	2.30	22.0	13.0	4.19	0.002

SE=Pooled SE.

^a Measures for NANI pigs for each plant and overall differ significantly from controls at $P < 0.05$.

1991). Therefore, the objective of this study was to assess the health of NANI and control pigs by looking at a range of measures, including complete blood chemistry, anatomy, and pathology.

2. Materials and methods

Data were collected from NANI and control pigs at each of five plants (A, B, C, D and E) in the USA. The five different plants were located at different geographical locations in the lower to upper midwestern USA. Daily animal capacity at the plants ranged from about 10,000 to 20,000 pigs per day. Approximately 15–20% of all pigs in the USA are processed at the five plants used in the present study.

NANI pigs were tattooed in the stressor pen (the pen where NANI pigs are held prior to processing) for identification once they were on the processing line. Control pigs were selected on the processing line; once a NANI pig was observed on the processing line the next pig that came along was allocated as a control pig. All control pigs were ambulatory sound during unloading at the plant. At plants A, B, C, and D control and NANI pigs were not necessarily from the same truck load or farm, due to the processing regime at the plants. However, all pigs from plant E did originate from only one farm.

2.1. Blood analysis

Blood was collected from NANI ($n=110$) and control ($n=98$) pigs for analysis of hematology and blood chemistry at four plants (B, C, D and E) and analysis was performed by the Department of Veterinary Pathology, Iowa State University, Ames, IA. Blood was not collected at plant A due to limited resources at the time. Ten milliliters of whole blood were collected over EDTA and 10 mL were collected without anticoagulant for serum. Whole blood was analyzed using the ADVIA®120 hematology system (The Jackson Laboratory, Bar Harbor, ME) for total white blood cell (WBC) counts, red blood cell (RBC) counts, platelet, neutrophil, neutrophil band, lymphocyte, eosinophil, basophil, and monocytes counts, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentrations (MCHC), red blood cell distribution width (RDW), nucleated RBC, plasma protein, and fibrinogen con-

centrations. Serum samples were analyzed using the Roche/Hitachi 912 (Roche Diagnostics, Basel, Switzerland) for blood urea nitrogen (BUN), creatinine, glucose, total protein, albumin, aspartate aminotransferase (AST), creatine kinase (CK), alkaline phosphatase (Alk Phos), gamma-glutamyl transpeptidase (GGT), total bilirubin, and haemolytic index. At plant E (only), serum was also collected from NANI ($n=52$) and control ($n=46$) pigs to determine the viremic status of these pigs. Isolation and titers for porcine reproductive and respiratory syndrome (PRRS), swine influenza virus (SIV) sub-types H1N1 and H3N2, PCV II and *Mycoplasma hyopneumoniae* were measured using ELISA (Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA).

At plants D and E, serum samples were assayed for cortisol using enzyme immunoassay kit (Assay designs, Ann Arbor, MI). Intra- and inter-assay CV were 4.1% and 2.7%, respectively.

2.2. Pathology

Internal organs of NANI ($n=124$) and control ($n=125$) pigs were inspected for pathology at four plants (B, C, D and E). Pathology of internal organs was not recorded at plant A due to limited resources at the time. The same veterinarian observed and scored the internal organs of pigs used in this study at each of the four plants. Stomachs were scored as empty, full, and presence of ulcers. Ulcers were scored as 0 (no signs of ulceration), 1 (minor) or 2 (severe). Livers were assessed for liver damage. Livers were scored as 0 (no signs of damage), 1 (minor) and 2 (severe). A zero to two scale was used to assess the severity of ulcers and liver damage as this was determined adequate to cover the range of pathologies observed in these animals and to make a distinction between healthy and diseased animals. Rhinitis was recorded in pigs processed at two of the plants (C and D). The presence of rhinitis was scored from 0 to 4; with 0 indicative of no rhinitis and 4 indicative of severe signs of rhinitis. Lungs were examined and the percent of total lung volume affected with consolidation was visually estimated.

2.3. Alveolar macrophage isolation

Pulmonary alveolar macrophages were collected by means of bronchoalveolar lavage (BAL) at four plants (B, C, D and E). Pulmonary alveolar macrophages were not collected at plant A due to limited resources at the time. Fifty milliliters of sterile

Table 4
Blood chemistry of non-ambulatory, non-injured (NANI) and control pigs averaged over four processing plants in the United States

Measure	NANI	Control	SE	P-value		
				Trt	Plant	Trt*Plant
<i>N</i>	110	98				
WBC, 10 ³ /μL	22.1	18.3	0.82	0.001	0.001	0.470
RBC, 10 ⁶ /μL	8.4	8.6	0.17	0.346	0.000	0.011
Hemoglobin, gm/dl	14.9	15.7	0.27	0.049	0.000	0.025
Hematocrit, %	50.1	52.3	1.03	0.126	0.000	0.002
MCV, fl	60.0	71.6	10.12	0.419	0.447	0.566
MCH, pg	17.9	18.3	0.20	0.227	0.864	0.931
MCHC, gm/dl	29.9	30.0	0.19	0.687	0.000	0.007
Platelets, 10 ³ /μL	176.6	201.2	15.32	0.263	0.000	0.169
Neutrophil, 10 ³ /μL	14.7	7.3	0.88	0.000	0.000	0.931
Band Neutrophil, 10 ³ /μL	0.3	0.1	0.06	0.029	0.000	0.032
Lymphocyte, 10 ³ /μL	6.6	10.0	0.53	0.000	0.527	0.156
N:L	3.0	0.8	0.28	0.000	0.033	0.168
Monocyte, 10 ³ /μL	0.5	0.4	0.07	0.418	0.031	0.178
Eosinophil, 10 ³ /μL	0.0	0.4	0.07	0.002	0.003	0.024
Basophils, 10 ³ /μL	0.0	0.0	0.01	0.044	0.879	0.972
RDW, %	18.0	17.7	0.19	0.152	0.000	0.027
Nucleated RBC	2.1	0.4	0.37	0.001	0.147	0.643
Plasma Protein, gm/dl	9.0	8.9	0.13	0.841	0.000	0.008
Fibrinogen, mg/dl	326.7	288.3	26.12	0.204	0.013	0.858
BUN, mg/dl	22.4	14.6	0.84	0.000	0.005	0.001
Creatinine, mg/dl	3.2	2.4	0.14	0.000	0.006	0.001
Glucose, mg/dl	117.8	131.1	6.52	0.152	0.000	0.015
Total protein, gm/dl	8.0	8.1	0.08	0.395	0.000	0.001
Albumin, gm/dl	4.5	4.8	0.07	0.006	0.000	0.317
AST, IU/L	689.0	201.9	60.23	0.000	0.000	0.656
CK, IU/L	4286.0	4075.6	558.35	0.791	0.000	0.207
ALK PHOS, IU/L	199.2	140.0	8.91	0.000	0.023	0.198
GGT, IU/L	40.8	35.2	1.76	0.025	0.003	0.013
Total Bilirubin, mg/dl	0.2	0.1	0.02	0.076	0.000	0.327
Hemolytic Index	50.7	59.4	8.59	0.477	0.000	0.001

SE=Pooled SE.

phosphate buffered saline (PBS) was added to lungs and massaged, and then PBS-lavage fluid was removed. Lavage fluid was centrifuged at 460 × *g* for 15 min. Supernatant was removed and frozen for later analysis. The cell pellet was washed in media and cytopins were made, fixed, stained with

Hema-3 staining system (Fisher Scientific, Houston, TX), and 100 cells per slide were visually counted under a light microscope. The different pulmonary alveolar macrophage sub-populations were characterized based on their morphology and staining pattern as previously described (Shellito and Kaltreider, 1984). Sub-populations 1 and 2, as well as 3 and 4, were combined as it was too difficult to differentiate between these sub-populations using light microscopy.

2.4. Feet analysis

Feet and legs of NANI (*n*=197) and control (*n*=192) pigs were examined for cracked hooves, swollen joints, or abscesses at all plants studied as an assessment of foot and leg health. The same person observed and scored the feet of all pigs used in this study. The hooves, pads and joints were inspected for lesions and the severity of the pathology was scored as 0 (no signs of damage), 1 (minor) and 2 (severe). A zero to two scale was used to assess foot and leg health as this was determined adequate to describe the range of feet and leg problems, furthermore the observer had limited time to make a more in depth inspection of the pigs feet and leg as the pigs passed on the line. Score 0: the pads were pink/white or normal, the hooves showed no cracks or chips, and the leg joints were normal with no swelling, puffiness or abrasions. Score 1: the pads showed a discolored “spot” less than 13 mm in diameter but no callused areas, or the hooves showed hairline cracks that extended from the bottom of the hoof but not to the hairline, and the joints were puffy but not abscessed or swollen. Score 2: the pads had large discolored spots (greater than 13 mm in diameter) or callused or swollen areas that extending over the pad, the hooves had large noticeable cracks wider and deeper than hairline that extend from the bottom of the hoof close to or to the hairline, hooves were broken or chipped, and/or the leg joints were abscessed or swollen and may have shown abrasions or skin breaks. All four feet were observed for feet and leg problems and the resulting score was the worst condition recorded for that particular pig. Gender was also recorded for NANI and control pigs.

2.5. Statistical analyses

A linear mixed-effects model using the MIXED procedure of SAS version 9.1.3 (SAS Inst., Inc., Cary, NC) was used to analyze blood hematology and chemistry, cortisol concentrations, macrophage sub-populations and virus titers. The main fixed effects were plant (five levels) and treatment (two levels). The interaction between plant and treatment were included. Chi-square analyses were used to analyze feet and internal organ pathology scores. Values were considered significant at *P*<0.05.

3. Results

Hoof and pad problems did not differ between control and NANI pigs, however there were differences between NANI and control pigs among the different plants (Table 1). NANI pigs had greater (*P*<0.01)

Table 5
Blood chemistry of non-ambulatory, non-injured (NANI) and control pigs from four different processing plants in the United States

Measure	Plant B			Plant C			Plant D			Plant E			P-value Trt*Plant
	NANI	Control	SE	NANI	Control	SE	NANI	Control	SE	NANI	Control	SE	
N	36	36		8	7		8	9		58	46		
WBC, 10 ³ /μL	26.1	20.4	1.28	22.3	17.1	1.96	17.3	15.3	1.99	22.6	20.3	1.12	0.470
RBC, 10 ⁶ /μL	9.1	8.6	0.27	7.6	8.1	0.41	9.0	9.4	0.42	7.7	8.4	0.24	0.107
Hemoglobin, gm/dl	16.4	15.8	0.43	13.6	14.3	0.64	16.1	17.3	0.65	13.5	15.3 ^a	0.73	0.025
Hematocrit, %	57.0	52.2 ^a	1.64	43.5	46.3	2.45	53.8	57.5	2.49	46.1	53.3 ^a	1.40	0.002
MCV, fl	62.4	103.9	16.11	57.3	57.7	24.11	59.8	61.1	24.55	60.4	63.7	13.82	0.566
MCH, pg	18.0	18.5	0.32	17.9	17.9	0.47	18.0	18.4	0.48	17.8	18.4	0.27	0.931
MCHC, gm/dl	28.9	30.2 ^a	0.31	31.2	31.1	0.46	30.0	30.0	0.47	29.6	28.8 ^a	0.26	0.007
Platelets, 10 ³ /μL	237.9	310.9	19.46	158.7	179.6	29.12	133.4	113.1	30.57	.	.	.	0.169
Neutrophil, 10 ³ /μL	18.4	11.6	1.40	13.7	4.8	2.09	10.5	4.0	2.13	16.1	8.8	1.20	0.931
Band Neutrophil, 10 ³ /μL	0.1	0.1	0.07	0.2	0.0 ^a	0.11	0.7	0.3	0.11	.	.	.	0.032
Lymphocyte, 10 ³ /μL	7.0	8.2	0.84	7.5	10.5	1.25	5.8	10.6	1.28	6.2	10.8	0.72	0.156
N:L	3.2	1.6	0.45	2.3	0.5	0.68	2.3	0.4	0.69	4.3	0.9	0.39	0.168
Monocyte, 10 ³ /μL	0.7	0.3 ^a	0.11	0.8	0.7	0.16	0.4	0.2	0.16	0.3	0.5 ^a	0.09	0.178
Eosinophil, 10 ³ /μL	0.0	0.2	0.11	0.1	1.0	0.17	0.0	0.2 ^a	0.17	0.0	0.1	0.10	0.024
Basophils, 10 ³ /μL	0.0	0.0	0.02	0.0	0.1	0.03	0.0	0.0	0.03	0.0	0.0	0.02	0.972
RDW, %	19.2	18.1 ^a	0.30	16.7	16.9	0.45	18.3	17.2	0.46	18.1	18.5	0.26	0.027
Nucleated RBC	0.9	0.1	0.53	2.5	0.3	0.80	3.6	1.0	1.04	1.5	0.1	0.46	0.643
Plasma Protein, gm/dl	9.7	9.0 ^a	0.21	7.6	8.6	0.31	9.5	9.0 ^a	0.34	9.0	9.1	0.18	0.008
Fibrinogen, mg/dl	286.7	342.9	40.52	233.3	157.1	60.63	312.5	500.0	65.82	286.7	340.7	34.75	0.858
BUN, mg/dl	20.8	13.3 ^a	1.02	17.9	14.0 ^a	2.24	29.9	13.6	2.10	21.0	17.7 ^a	0.87	0.001
Creatinine, mg/dl	3.6	2.2 ^a	0.17	2.8	2.1	0.37	3.7	3.1	0.35	2.6	2.5	0.14	0.001
Glucose, mg/dl	96.4	100.9	7.92	133.1	94.9 ^a	17.38	153.1	214.1	16.32	88.5	114.4 ^a	6.90	0.015
Total protein, gm/dl	8.1	7.9	0.10	7.5	8.4	0.22	8.7	8.2 ^a	0.21	7.5	7.8 ^a	0.09	0.001
Albumin, gm/dl	4.8	4.9	0.08	4.0	4.4	0.18	4.7	4.9	0.17	4.5	4.8	0.07	0.317
AST, IU/L	555.7	82.6	73.30	359.8	70.6	160.87	1206.3	570.1	151.05	634.4	84.4	62.63	0.656
CK, IU/L	4061.0	4790.0	853.35	4426.0	2800.4	1095.02	780.5	2495.0	1619.41	7876.5	6217.0	270.17	0.207
ALK PHOS, IU/L	215.7	152.2	10.84	160.0	96.3	23.79	228.5	146.7	22.33	192.7	164.7	9.26	0.198
GGT, IU/L	39.8	41.4	2.14	36.3	28.0	4.70 ^a	44.8	26.6	4.41	42.4	44.8	1.83	0.013
Total Bilirubin, mg/dl	0.2	0.1	0.02	0.1	0.1	0.05	0.2	0.2	0.05	0.1	0.0	0.02	0.327
Hemolytic Indice	21.1	4.8	10.46	59.5	6.1	22.95	93.5	200.4	21.55	28.6	26.0	8.93	0.001

SE=Pooled SE.

^a Measures for NANI pigs for each plant and overall differ significantly from controls at $P<0.05$.

percent of pad problems compared with control pigs at plant A. The percentage of total feet and leg injuries was greater ($P<0.05$) in NANI compared with control pigs at plant E. Conversely, the percentage of hoof problems was less ($P<0.01$) among NANI than control pigs at plant A and the number of hoof problems with a score of 2 was lower ($P<0.01$) in NANI compared with control pigs at plant B. The percentage of severe hoof and pad problems were greater ($P<0.01$) in NANI compared with control pigs at plant A and E. Conversely, severe foot and pad problems were less ($P<0.01$) among NANI than control pigs at plant B.

Gender had an impact on the percent of NANI pigs at one plant (Table 1). At plant D, a higher ($P<0.05$) percentage of barrows were NANI than were gilts.

Internal organs were inspected for gross signs of disease. Overall, signs of disease did not differ between NANI and

control pigs, however there were differences in pathology scores for different organs between NANI and control pigs within the plants (Table 2). The incidence of ulcers was greater ($P<0.05$) in NANI pigs compared with controls at plants B and D; however, at plant E, control pigs displayed a greater ($P<0.01$) percentage of ulcers than NANI pigs. Rhinitis scores were measured in two of the five plants; at plant D, the average rhinitis score was greater ($P<0.01$) among control than NANI pigs, but no differences were observed at plant C. At plants B and C the total percentage of liver problems was greater ($P<0.05$) among NANI than control pigs. The percentage of empty stomachs was greater ($P<0.05$) among NANI compared with control pigs at plants C and D. Conversely, at plant E the percentage of empty stomachs was greater ($P<0.01$) among control than NANI pigs. Lung consolidation was not different between NANI and control pigs at any of the plants.

Table 6
Virus isolation and titers in serum of non-ambulatory, non-injured (NANI) and control pigs from one processing plant

Measures	NANI	Control	<i>P</i> -value
<i>N</i>	52	46	
Titers			
Porcine reproductive and respiratory syndrome	1.355	1.368	
<i>Mycoplasma hyopneumoniae</i>	0.415	0.402	
Swine influenza sub-types H1N1	0.410	0.650	0.01
Swine influenza sub-types H3N2	0.388	0.604	0.01
Porcine circovirus II	6.203	10.819	0.001
% Positive viremia			
Porcine reproductive and respiratory syndrome	88.5	93.5	
<i>Mycoplasma hyopneumoniae</i>	40.4	47.8	
Swine influenza sub-types H1N1	53.8	34.8	0.01
Swine influenza sub-types H3N2	51.9	26.1	0.01
Porcine circovirus II	100.0	100.0	

Overall, alveolar macrophage sub-populations did not differ between NANI and control pigs (Table 3). However at plant C, alveolar macrophage sub-population 5 was greater ($P < 0.001$) among control than NANI pigs.

Blood chemistry differed between NANI and control pigs (Table 4). White blood cells counts, the percentage of neutrophils and basophils, the neutrophil to lymphocyte ratio, nucleated red blood cells, and AST and Alk Phos concentrations were greater ($P < 0.05$) among NANI than control pigs (Table 4). Conversely, the percentage of lymphocytes and the concentration of albumin were less ($P < 0.05$) among NANI pigs than controls (Table 4). Gamma-Glutamyl Transpeptidase, creatinine, BUN, RDW, and neutrophil bands were greater ($P < 0.05$) among NANI compared to control pigs at one or more of the processing plants (Table 5). Conversely, total protein, Hg, glucose, eosinophils, and the hemolytic index were lower ($P < 0.05$) among NANI pigs compared with their controls, in one or more of the processing plants (Table 5). Cortisol concentrations did not differ between NANI (80.0 ± 1.83) and control (80.5 ± 1.87) pigs.

Virus titers were measured in the serum of NANI and control pigs at plant E only (Table 6). Titers to SIV sub-types H1N1 and H3N2 and PCV II were less ($P < 0.01$) among NANI compared with control pigs. However, more NANI pigs were positive for SIV sub-types H1N1 and H3N2 compared with control pigs ($P < 0.01$).

4. Discussion

Hoof and pad problems did not differ between control and NANI pigs, however there were differences between NANI and control pigs' feet among the dif-

ferent plants. Despite the fact that there was no overall difference in feet and hoof problems between NANI and control pigs, over 50% of the pigs had hoof problems or pad problems of some kind and over 30% of these problems were severe. Therefore, it is apparent that hoof and pad problems in slaughter weight pigs are a problem and a welfare concern that needs to be addressed even though it does not seem to directly impact the occurrence of NANI pigs, except in certain situations (ex., Plant A and E).

A higher percentage of barrows were identified as NANI compared with gilts, in one of the plants studied. The gender of pigs being transported in trailers to the processing plant has been shown to significantly affect the percentage of NANI pigs during transport (unpublished data from this lab). The literature regarding the effect of gender on transport loss is limited. One possibility is that barrows may fight more than gilts during transport. Transporting of pigs usually involves the mixing of unfamiliar pigs, which often results in fighting to establish new dominance orders (McGlone, 1985). A British survey on transport of finishing pigs found that two thirds of pigs dead on arrival had been involved in fights (Sains, 1980). Fighting in pigs can cause stress, possibly resulting in fatigue (Warriss, 1995). Therefore, fighting of pigs during transport could possibly result in a higher incidence of NANI pigs. However, the frequency of aggressive behavior after mixing of young pigs did not differ between young gilts and castrated-males (McGlone, 1985). Fighting during transport could result in pigs becoming fatigued and consequently going down, but it is unlikely that this explains the gender effect on the incidence of NANI pigs. Therefore, more research is needed in this area to answer this question.

Low energy levels among pigs have been suggested as a possible cause of pigs becoming NANI during or after transport. However, in the present study less than 30% of NANI pigs had empty stomachs (or 70% had feed in their stomachs) and this was similar among control pigs. Blood glucose goes down with food deprivation (Bertol et al., 2005), but in the present study glucose concentrations were within the normal range for both NANI and control pigs (Carr, 1998; Mersmann and Pond, 2001). The presence of food in the stomach of most NANI pigs in combination with average blood glucose concentrations measured in NANI pigs make it unlikely that NANI pigs went down due to fatigue caused by low energy stores, however measurement of muscle glycogen levels in future studies may give a better indication of an animals energy reserves.

Pathology of internal organs was recorded to determine if disease was a potential cause for pigs becoming

NANI. Overall, there were no differences in the pathology of the liver, stomach, or lungs between NANI and control pigs, but there were differences between NANI and control pigs at particular plants. Therefore, pigs do not appear to go down due to a single disease. Rather, at one plant, pigs becoming NANI may be associated with ascarid infection, another may have an active (but not a resolved) respiratory disease, and another may have feet and leg problems (or other health problems).

Cortisol concentrations were measured in NANI and control pigs at two of the five plants. Cortisol concentrations did not differ between NANI and control pigs, however cortisol concentrations were elevated in all pigs compared with baseline cortisol concentrations of age matched non-stressed pigs (unpublished data from this lab), suggesting that all pigs were experiencing stress prior to harvesting. Measuring cortisol at one time point as an indicator of stress is not optimal. It would have been preferable to compare the cortisol response to transport in control vs. NANI pigs, however this was beyond the scope of this study. Hematology measures differed significantly between NANI and control pigs. The neutrophil to lymphocyte ratio was significantly higher in NANI pigs than controls. Acute 4 h transport stress has been shown to increase the neutrophil to lymphocyte ratio in pigs (McGlone et al., 1993), hence the high cortisol concentrations in control and NANI pigs suggest that all pigs are stressed at harvesting but the increase in the neutrophil to lymphocyte ratio measured in NANI pigs may be an indication that these pigs are experiencing even more stress than controls.

The percentage of lymphocytes were reduced in NANI compared with control pigs. Transient leukopenia and lymphopenia are often induced in pigs that have been infected with the PRRS virus, but lymphocyte numbers return to normal within eight to 14 days post-inoculation (Nielsen and Bøtner, 1997; Toepfer-Berg et al., 2004; Sutherland et al., 2007). Non-ambulatory, non-injured pigs did not have a greater percentage of pigs positive for PRRS compared with control pigs at plant E. However, reduced lymphocytes in NANI pigs in the present study may suggest that these animals are experiencing an active infection. For example, NANI pigs may have been infected with the PRRS virus less than eight days prior to being shipped to the processing plant. At plant E, serum titers to common pig respiratory viruses were measured. Titers to one or more respiratory viruses were measured in 100% of the pigs, in both NANI and control pigs. However, it was not determined if these titers were formed in response to an active infection or from a past infection. NANI pigs had significantly lower titers to SIV sub-types

H1N1 and H3N2 and PCV II compared with control pigs, but a higher percentage of NANI pigs were positive for SIV sub-types H1N1 and H3N2 compared with control pigs. It is unclear why NANI pigs would have lower viral titers compared with controls, but one possibility is that NANI pigs may be immunosuppressed for whatever reason. The combination of leucopenia, positive titers to various respiratory viruses and lower viral titers compared with control pigs are indications that the health of these pigs is compromised.

Inflammatory processes are characterized by increased leukocyte counts, fibrinogen and total protein concentrations, and reduced hematocrit, Hg, and albumin (Odink et al., 1990). In the present study, leukocytes were increased and Hg and albumin concentrations were reduced among NANI pigs, suggesting that these pigs may have been experiencing some form of inflammatory response. Albumin concentrations have also been shown to be reduced in pigs with abscesses (Smeets et al., 1990; Elber et al., 1992). Changes in leukocyte percentages and albumin concentrations in NANI pigs suggest that these pigs were experiencing some sort of active infection whether due to viral infection or inflammation which may be a contributing factor for them going down during transport.

Blood chemistry measures were significantly different between NANI and control pigs in the present study. Creatine kinase is released from muscle fibers in response to intense muscle exertion or tissue damage. Values of CK measured in this study were in the upper limit of the normal range for CK (Carr, 1998) for both NANI and control pigs. Creatine kinase concentrations were reported to increase in market weight pigs during transport to the packing plant (Elbers et al., 1991) and in pigs kept at stocking densities lower than 0.5 m²/100 kg during transport (Barton-Gade and Christensen, 1998; Warriss, 1998). Therefore, elevated CK concentrations measured in NANI and control pigs was probably due to tissue damage caused by muscle exertion as a result of transport to the processing plant rather than a causative factor in NANI pigs going down during transport.

Creatinine is a waste product produced when muscle cells are broken down. Blood urea nitrogen is a waste product in the blood caused from the breakdown of protein. Creatinine and BUN can also be indicators of kidney dysfunction. In the present study, creatinine concentrations in pigs were above the normal range (Carr, 1998; Mersmann and Pond, 2001) and higher among NANI than control pigs. The high creatinine and BUN concentrations among NANI pigs may reflect possible kidney dysfunction in these animals, which could possibly lead to these pigs becoming NANI during transport. Alternatively, the higher creatinine and

BUN may indicate that NANI pigs have more muscle and protein break down than control pigs.

Aspartate aminotransferase is a hepatic enzyme released in the blood when certain organs or tissues, particularly the heart or liver, are injured. Alkaline phosphatase is an enzyme present within the liver, bone, kidneys, and intestines and leaks into the blood when cells from these organs are destroyed. Increased AST and Alk Phos concentrations are an indicator of liver damage, or an indicator of bone damage. Aspartate aminotransferase and Alk Phos were higher among NANI than control pigs in the present study. Furthermore, the presence of liver problems were greater among NANI pigs than control pigs at two of the processing plants. Increased concentrations of these two enzymes in NANI pigs in combination with liver damage possibly associated with ascarid infection indicate that these animals may have liver damage. Increased AST and Alk Phos concentrations in NANI pigs, may also be explained by NANI pigs having slight bone injuries or fractures. Fractures were recorded in 26% of market weight pigs which died during transport in Canada (Clark, 1979). The elevated AST and Alk Phos are consistent with NANI pigs having a hairline fracture, or a bruised bone (but not a compound fracture, which would be obvious and classified as a non-ambulatory injured pig). Increased AST and Alk Phos concentrations in NANI pigs suggest that these pigs have liver problems, slight bone injuries or both which could result in these pigs going down during transport.

Total protein and albumin concentrations are markers for protein homeostasis, which increase with dehydration. Albumin concentrations usually parallel the total protein concentrations. Total protein was high, but still within the normal range for growing pigs, but albumin was slightly above the normal range (Carr, 1998; Mersmann and Pond, 2001). Furthermore, total protein was higher in control pigs at two plants and albumin was higher in control pigs overall, suggesting that dehydration was not a causative factor of NANI pigs in the present study.

Blood chemistry analyses and pathology indicate large differences between NANI and controls pigs. Severe energy loss as measured by blood glucose and stomach content was not a cause of pigs becoming NANI. The present study did not find one determining factor for the cause of NANI pigs. Pigs probably become NANI due to one or a combination of factors. Non-ambulatory, non-injured pigs generally had one or more of the following conditions: feet and leg problems, an active infection, ulcers, liver damage, subtle bone injury, and were in a catabolic state.

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