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EFFECT OF TRANSPORT STRESS ON PERIPHERAL BLOOD LYMPHOCYTE SUBSETS AND Th CYTOKINES IN PIGS

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In order to investigate transport stress on porcine peripheral blood lymphocyte subsets and Th cytokines, blood samples were collected from pigs before and after transport. Creatine kinase (CK), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), glucose, and cortisol in the serum were measured. The number of leukocytes and lymphocytes, percentages of lymphocyte subsets, as well as Th cell cytokines level and their mRNA expression were detected, respectively. After transport, the level of CK, glucose and interleukin (IL)-4 increased significantly ($p<0.01$), LDH, Th memory cells, natural killer and interferon (IFN)-γ increased significantly ($p<0.05$), cortisol, number of leukocytes and lymphocytes decreased ($p<0.01$), percentages of $\gamma\,\delta$ T cells, naïve Th cells and cytotoxic T lymphocytes decreased significantly ($p<0.05$). The mRNA expressions IL-2 and IFN-γ were down regulated, $p<0.01$ and $p<0.05$, respectively. While IL-4, IL-6, and IL-10 were up regulated, but only IL-10 displayed a highly significant difference ($p<0.01$). These data suggested that transport could cause immune suppression in pigs, which influences cellular immunity more than humoral immunity, and humoral immunity may play an important role in transport stress. Proper measures should be taken to reduce susceptibility of infection after transport.

Key words: lymphocyte, peripheral blood, pigs, Th cytokines, transport stress

INTRODUCTION

Transport stress is a complex stress, in which many stressors are involved, such as upload and offload, crowding, rocking, noise, temperature, food and water deprivation (Earley *et al*., 2010). Stress response occurs easily in pigs due to transport (Von Borell, 2001), causing pale, soft, and exudative meat (Pérez *et al*., 2002). Severe stress may even result in death, with mortality rates reported up

to 0.1%-1.0% (Allen *et al*., 1974; Averós, 2008; Warriss *et al*., 1989). The serum concentrations of creatine kinase (CK) (Hong *et al*., 2007), lactate dehydrogenase (LDH), glucose, and cortisol can be affected by transport stress (Yoshioka *et al*., 2004); transport stress can also increase amounts of heat shock protein (HSP)70 and Hsp70 mRNA expression in the heart and kidney tissues of transported pigs (Hong *et al*., 2007).

Stress inhibits the immune system function of animals, which can increase morbidity (Salak-Johnson and McGlone, 2007). Immune suppression caused by transport stress has also been reported (Frank *et al*., 1984). Studies have shown that transport stress increases the leukocyte number, but decreases the lymphocyte number in peripheral blood (Dalin *et al*., 1993), and changes the serum levels of IL-2, IL-6, and IL-10, as well as the expression of their receptors in the thymus (Qiongxia, 2011), which indicates that transport stress may have an effect on the porcine immune system. However, there is limited knowledge about the effects of transport stress on lymphocyte subsets and Th cell cytokines in porcine peripheral blood.

Effects of transport stress on the immune system are an important problem during new stock introduction and transfer of groups to new locations. The age at which introduction or group transferring of pigs occurs in China is almost the same with grown and fattening pigs. Therefore, grown and fattening pigs were employed to study the effects of transport stress on the porcine immune system, by analyzing total lymphocyte number and subsets, Th cell cytokines IFN-γ, IL-4, and IL-6, as well as the mRNA expression of Th cell cytokines, aiming at providing more detail of porcine immunity response to transport stress.

MATERIALS AND METHODS

Animals and transport

Five castrated male pigs (Sherlock and Landrace cross-breed) with mean body weight of 105.2±2.4 kg from one farmhouse in a Peking suburb were randomly selected for the experiment. All pigs were loaded on the truck under commercial transport conditions. The transport distance was about 130 km lasting about 130 min with a speed of 60-70 km/h, which took place in July in the morning from 9:40 am to 11:50 am. The weather was cloudy and the temperature was between 26°C-29°C.

All experimental protocols were approved by the Committee for the Care and Use of Experimental Animals at China Agricultural University.

Blood collection and treatment

Before loading and 2 hrs after unloading, 10 mL of blood was collected from the precaval vein. Each blood sample was divided into two tubes equally with/without heparin sodium anticoagulant (Sanli, Hunan, China), and then kept in an icebox before arriving at the laboratory. Serum was collected from blood samples without added anticoagulant after being centrifuged at 4°C and 3000 x g for 15 min, which was stored at -20°C for further analysis. Heparinized blood

samples were used for complete blood counts, flow cytometry and extraction of total RNA.

Tests of biochemistry and cortisol

Concentrations of serum CK, LDH, alkaline phosphatase (ALP), and glucose were detected by an automated biochemical analyzer (TBA-40FR, Toshiba, Tokyo, Japan), in which performance rate method was used for CK, LDH, and ALP and glucose oxidase method was used for glucose. The level of cortisol in the serum was measured by immunoassay analyzer (MiniVidas, biomérieux, Fance) with radiation immunofluorescence method.

Determination of number of leukocytes and lymphocytes and lymphocyte subsets in peripheral blood

Leukocyte and lymphocyte numbers were counted by an automated blood cell analyzer (Mek-7222K, Nihon Konden, Tokyo, Japan) with electric-resistivity method.

Flow cytometry was employed to investigate the difference of porcine lymphocyte subsets in peripheral blood between, before, and after transport, which was represented as percentage. Triple-color flow cytometry with CD3, CD4, and CD8 was used to analyze the lymphocytes subsets in porcine peripheral blood lymphocytes (PBL) with monoclonal antibodies anti-pig CD3e-SPRD (PPT3, BD Biosciences, New York, NY, USA), anti-pig CD4a-FITC (74-12-4, BD Biosciences, New York, NY, USA), and anti-pig CD8a-PE (76-2-11, BD Biosciences, New York, NY, USA) according to the methods used in our previous study (Jiefeng *et al*., 2011). Briefly, 200 μL heparinized blood samples were labeled simultaneously with the anti-pig monoclonal antibodies mentioned above for 30 min in dark at room temperature. Appropriate negative controls and labeled single antibodies were prepared at the same time. All samples were added to 1000 μL RBC lysate solution and incubated in dark at room temperature for 15 min, then centrifuged at 4ºC and 1500 x g for 5 min. The supernatant was discarded. This procedure was repeated if necessary. The sample was washed with 1 mL cold PBS and centrifuged at 4°C and 1500 x g for 5 min and re-suspended with 0.5 mL cold PBS to ensure that each sample contained at least 1 000 000 cells/mL. For each sample, 10 000 cells were analyzed using a FACS Calibur™ flow cytometer (BD Biosciences, New York, NY, USA). $CD3^+CD4^-CD^-$ indicates γ δ T cell, $CD3^+CD4^+CD8^-$ indicates naïve Th cell, $CD3^+CD4^-CD8^+$ indicates cytotoxic lymphocytes (CTL), $CD3^+CD4^+CD8^+$ indicates Th memory cells, and $CD3^-CD4^-CD8^+$ indicates natural killer (NK) cells.

Determination of serum cytokines by ELISA

Concentrations of serum IFN-γ, IL-4, and IL-6 were measured by commercial ELISA kits (R & D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions; the assay ranges were 5~2,000 pg/L, 1.8~80 ng/L, and 20~6,000 ng/L, respectively. Each sample optical density was read at 450 nm using a microliter plate reader (Synergy HT, Bio-Tek, Winooski, VT, USA) within

10 min. A standard curve was constructed by plotting the absorbance obtained for each reference standard against its concentration on a linear graph, with absorbance values on the (x) axis and concentration on the (y) axis.

Expression of cytokines mRNA

Extraction of total RNA and reverse transcription. Total RNA was isolated from peripheral blood lymphocytes, which was separated from 1000 μL peripheral blood by a lymphocyte separation medium (solarbio, Beijing, China) followed by the use of phenol and guanidine isothiocyanate-based TRIZOL reagents (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The concentration and purity of isolated RNA were determined by a spectrophotometer (SmartSpec plus, BIO-RAD, Hercules, CA, USA) based on the OD_{260}/OD_{280} ratio. The mRNA was reversely transcribed by the following two procedures: firstly, 1.0 μg mRNA was isolated from each blood sample, and 1.0 μL Oligo-dT18 (0.5 μg/μL) and RNase-free water was added to a 13 μL reaction system, which was incubated at 70°C for 5 min. Secondly, 1.25 μL dNTPs (10 mM), 0.5 μL RNase inhibitor (40 U/μL), 1.0 μL M-MLV transcriptase (200 U/μL), 5.0 μL M-MLV RT reaction buffer (5×) (Promega, Madison, WI, USA) and 4.25 μL RNase-free water were added into the first reaction system, then incubated in 42°C for 60 min. The reverse transcription products (cDNA) were stored at -20°C for quantitative RT-PCR.

Semi-quantitative real-time PCR. The mRNA expressions of IFN-γ, IL-2, IL-4, IL-6, and IL-10 were semi-quantitatively determined by RT-PCR. Semi-quantitative RT-PCR analysis was carried out using the DNA Engine Mx3000P® fluorescence detection system against a double-stranded DNA-specific fluorescent dye (Stratagene, La Jolla, CA, USA) according to optimized PCR protocols. β-actin was amplified at the same time with the target genes as the control. The cDNA was subjected to RT-PCR using the primer pairs and reaction condition as listed in Table 1. A 20 μL reaction system was used in the RT-PCR, containing 10 μL of SYBR Green qPCR mix, 0.3 μL of reference dye, 1 μL of each primer (both 10 μmol/L), and 1 μL of cDNA template. Expression levels were determined by the threshold cycle (CT) method as described by the manufacturer of the detection system. This method was applied to each gene by calculating the expression $2^{-\Delta\Delta CT}$, in which ΔΔCT was expressed as: $(CT_{gene} - CT_{\beta\text{-actin}})_{\text{Post-transport}} - (CT^{gene} - CT_{\beta\text{-actin}})_{\text{Pre-transport}}$.

Statistical analysis

All data are expressed as mean ± SD (standard deviation). SPSS software (version 11.5; SPSS Inc., Chicago, IL, USA) was employed for statistical analysis including independent-sample T-tests, in which $p<0.05$ was considered significant, $p<0.01$ was considered highly significant.

Table 1. Primers employed in the RT-PCR for the study

Description	Primer sequences		Parameters of RT-PCR	Amplicon (bp)	Accession NO
IL-2	Forward:	CAGCTCTTGTGTTGC ATTGCA	95°C for 15 min; 40 cycles of 95°C for 10 s, 62°C for 30 s; 95°C for 60 s, 62°C for 30 s, 95°C for 30 s	101	NM213861
	Reverse:	AGCAATGGCTCCAGT TGTTTCT			
IL-4	Forward:	TCTCACCTCCCAACT GATCC	95°C for 15 min; 40 cycles of 95°C for 10 s, 62°C for 30 s; 95°C for 60 s, 62°C for 30 s, 95°C for 30 s	201	NM_214123
	Reverse:	AAGGTTTCCTTCTCCG TCGT			
IL-6	Forward:	TTCACCTCTCCGGAC AAAAC	95°C for 15 min; 40 cycles of 95°C for 10 s, 56°C for 30 s; 95°C for 60 s, 56°C for 30 s, 95°C for 30 s	154	NM_214399
	Reverse:	TTTCTGCCATTTTTGG AAGG			
IL-10	Forward:	TAATGCCGAAGGCAG AGAGT	95°C for 15 min; 40 cycles of 95°C for 10 s, 56°C for 30 s; 95°C for 60 s, 56°C for 30 s, 95°C for 30 s	169	NM_214041
	Reverse:	TGGAGCTTGCTAAAG GCACT			
IFN-γ	Forward:	TCAAAGGAGCATGGA TGTGATC	95°C for 15 min; 40 cycles of 95°C for 10 s, 56°C for 30 s; 95°C for 60 s, 56°C for 30 s, 95°C for 30 s	101	NM_213948
	Reverse:	CCGGAATTTTAATCAG CTTTTCG			
β-actin	Forward:	GCGGCATCCACGAAA CTAC	95°C for 15 min; 40 cycles of 95°C for 10 s, 56°C for 30 s; 95°C for 60 s, 56°C for 30 s, 95°C for 30 s	312	NM_031144
	Reverse:	AGAAGCATTTGCGGT GGAC			

Primer sequences (5'–3') used for detection of mRNAs specific for cytokines and housekeeping genes by RT-PCR in pigs.

RESULTS

Changes of serum levels of creatine kinase, lactate dehydrogenase, alkaline phosphatase, glucose and cortisol

In the current experiment, the serum concentrations of CK and glucose increased significantly ($p<0.01$), LDH increased significantly ($p<0.05$), ALP also increased but not significantly ($p>0.05$) (Fig. 1A and B), and the serum level of cortisol decreased significantly ($p<0.01$) (Fig. 1C).

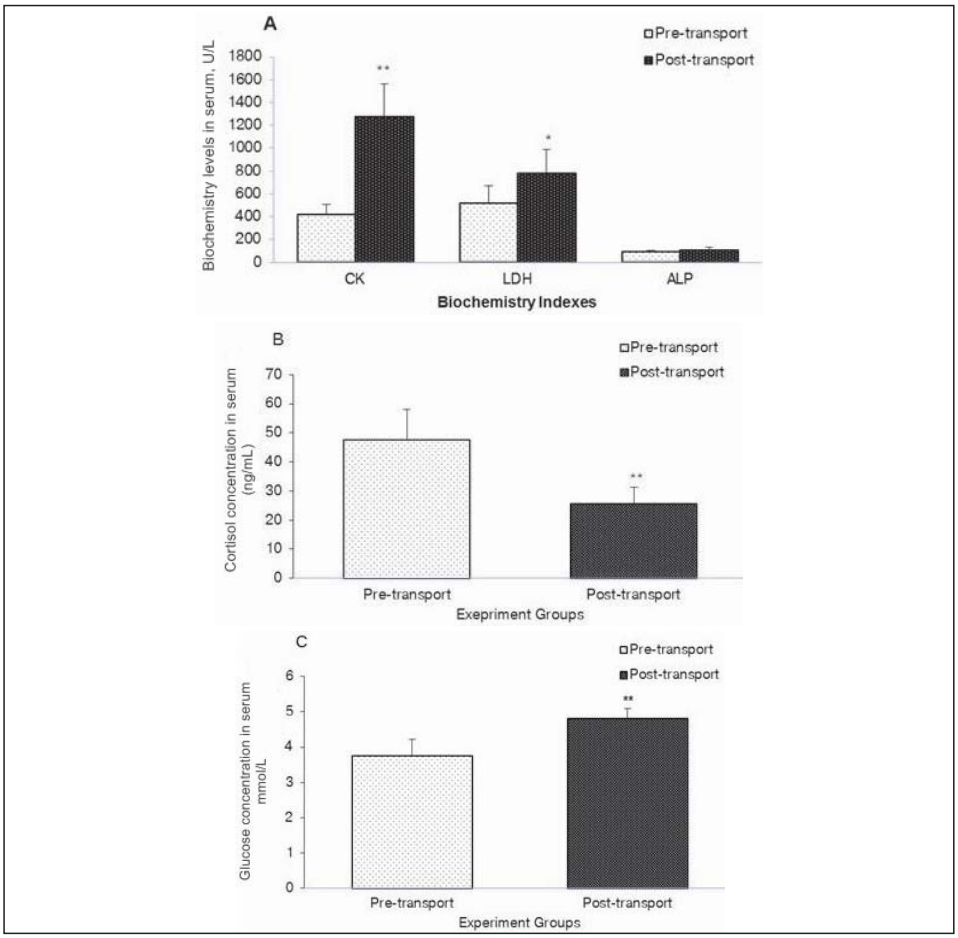

Figure 1. Changes of serum levels of biochemistry indices and cortisol

Mean of biochemical indexes and cortisol concentration in serum between pre-transport and post-transport. (A) Serum levels of creatine kinase (CK), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP), all of which increased after transport, $p<0.01$, $p<0.05$ and $P>0.05$, respectively. (B) Serum level of glucose, which increased significantly after transport, $p<0.01$. (C) Serum level of cortisol, which decreased significantly after transport, $p<0.01$. Error bars represent the mean as ± SEM. *: $p<0.05$; **: $p<0.01$

Changes of the number of leukocytes and lymphocytes, and percentages of lymphocyte subsets

The number of both leukocytes and lymphocytes in PBL decreased significantly after transport, ($p<0.01$; Fig. 2).

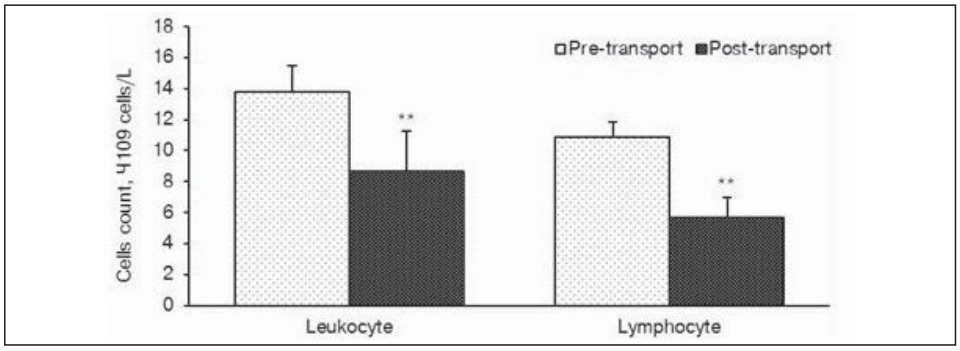

Figure 2. Number changes of leukocyte and lymphocyte in peripheral blood
Mean of leukocyte (LEU) and lymphocyte (LYM) numbers in porcine peripheral blood between pre-transport and post-transport ($x10^9$ cells/L). Both LEU and LYM in peripheral blood decreased significantly after transport, $p<0.01$, $p<0.01$, respectively. Error bars represent the mean as ± SEM. *: $p<0.05$; **: $p<0.01$

All of the percentages of lymphocyte subsets changed significantly post-transport when compared with pre-transport. γ δ T cells, naïve Th cells and CTL cells decreased significantly ($p<0.05$), and Th memory cells and NK cells increased significantly ($p<0.05$) (Fig. 3).

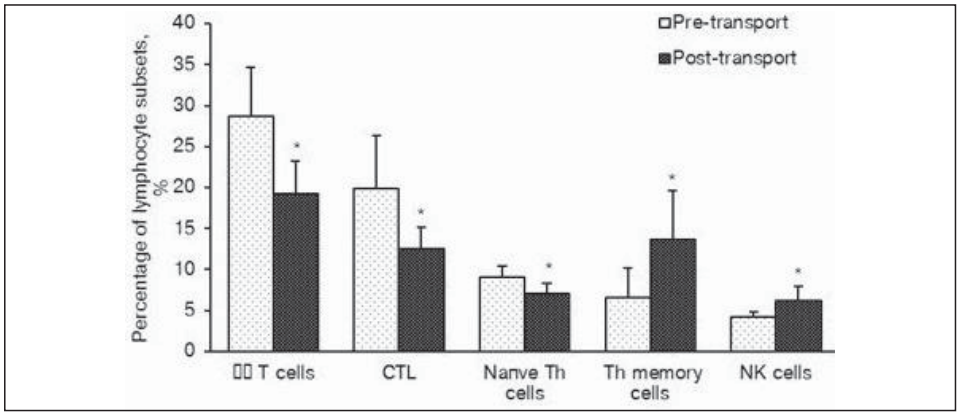

Figure 3. Percentage changes of lymphocyte subsets in peripheral blood
Mean of lymphocyte subsets in porcine peripheral blood between pre-transport and post-transport. As indicated in the figure, the difference of lymphocyte subsets were significant ($p<0.05$), in which percentages γ δ T cell, CTL, and naïve Th cell decreased, Th memory cell and NK cell increased. Error bars represent the mean as ± SEM. *: $p<0.05$; **: $p<0.01$

Changes of serum T helper cell cytokines

Serum concentrations of IFN-γ, IL-4, and IL-6 were detected before and after transport in the current study. All the concentrations of cytokines increased with different levels after transport. IFN-γ increased significantly ($p<0.05$; Fig. 4 A), and IL-4 increased highly significantly ($p<0.01$; Fig. 4 B), but there was no significant differences with IL-6 between before and after transport ($p>0.05$; Fig.4 C).

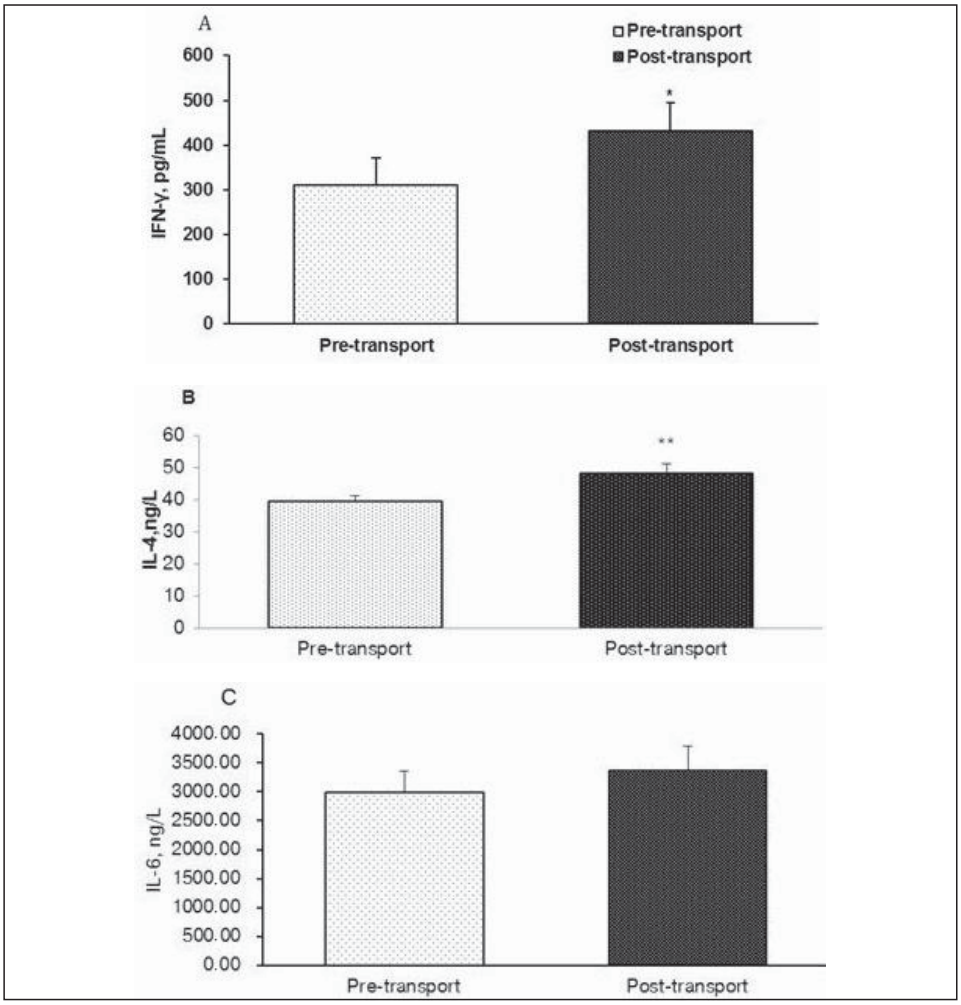

Figure 4. Serum concentrations of T helper cell cytokines in peripheral blood
Mean of major Th cell cytokines concentrations in serum. Interferon (IFN)-γ, IL-4 and IL-6 increased after transport. There were significant differences of IL-4 and IFN-γ between pre-transport and post-transport, $p<0.01$ and $p<0.05$, respectively. The difference of IL-6 between pre-transport and post-transport was not significant, $p>0.05$. Error bars represent the mean as ± SEM. *: $p<0.05$; **: $p<0.01$

mRNA expression of T helper cell cytokines

The mRNA expressions of IFN-γ, IL-2, IL-4, IL-6, and IL-10 were investigated by semi-quantitative RT-PCR. The mRNA expressions of IL-2 ($p<0.01$) and IFN-γ ($p<0.05$) were down regulated post-transport compared with pre-transport. In contrast, the mRNA expressions of IL-4, IL-6, and IL-10 ($p<0.01$), were up regulated after transport (Fig. 5).

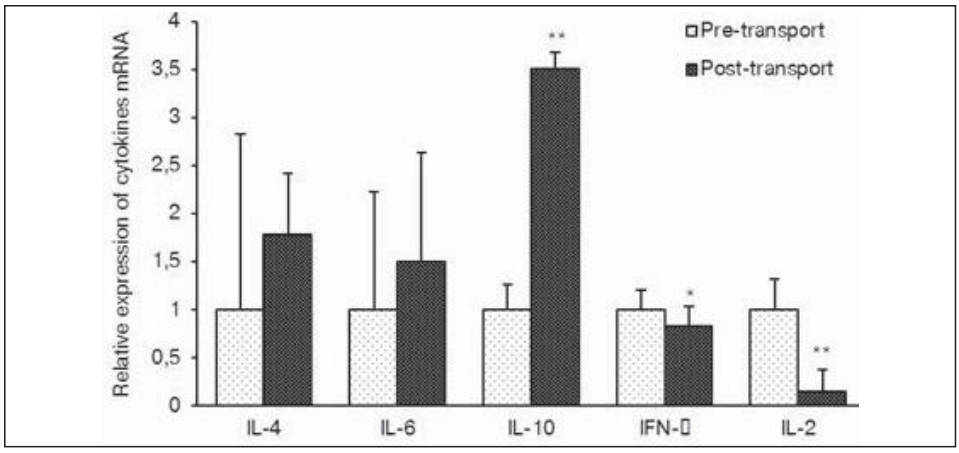

Figure 5. Relative mRNA expression of T helper cell cytokines
Relative mRNA expression of Th cell cytokines. The mRNA expression of humoral immune cytokines (IL-4, IL-6 and IL-10, $p<0.01$) up regulated after transport; the cellular immune cytokines (IL-2, $p<0.01$ and IFN-γ, $p<0.05$) down regulated after transport. *: $p<0.05$; **: $p<0.01$

DISCUSSION

Evaluation of porcine transport stress

Previous studies have shown that serum concentrations of CK, LDH, and glucose increase after transport (Bao, 2008; Deyi *et al*., 1996). Dynamic study of cortisol done by Dalin *et al*. (1993) showed that serum concentration of cortisol increased to peak level after 30 min of transport, decreased immediately after offload, and reached the lowest point at 4 h post transport. Investigations done by Bradshaw *et al*. (1996) also revealed a decrease of cortisol after transport. Similarly, a CK, LDH, ALP, and glucose increase and cortisol decrease after transport were found within the current study (Fig.1 A, B, and C), indicating that a stress response occured during transport.

Inhibition effect of transport stress on lymphocyte subsets

The number of leukocytes and lymphocytes in the peripheral blood is also used as a marker of immune status in transported animals (Obernier and Baldwin, 2006; Stanger *et al*., 2005). Transport stress has been shown to cause an increase

of leukocyte number and decrease of lymphocyte number in the peripheral blood of gilt (Dalin *et al*., 1993). The function of lymphocytes, and the production of cytokines, can be influenced by a variety of stressors (Meagher *et al*., 2007). T lymphocytes play a variety of important roles in immune regulation, inflammation, and protective immune response throu responses of lymphocyte subsets (Wilhelm *et al*., 2009). Our results showed that both leukocyte and total lymphocyte numbers decreased after transport, indicating the porcine immune status was suppressed. In addition, decreased percentages of $\gamma\,\delta$ T cells, naïve Th cells and CTL cells, and an increase in Th memory cells and NK cells were found after transport in our study (Fig. 3).

$\gamma\,\delta$ cells are thought to play an important role in innate and adaptive immune responses (Devilder *et al*., 2009; Pitard *et al*., 2008; Tian *et al*., 2006), including acting as antigen presenting cells by expressing major histocompatibility complex (MHC) II (Takamatsu *et al*., 2002), releasing cytokines/chemokines, having a cytotoxic function (Carding and Egan, 2002), attacking infected cells, and having immune memory functions (Takamatsu *et al*., 2006). Early activation of $\gamma\,\delta$ T cells can promote the production of IFN-γ, which improves the function of macrophages and NK cells, having an early antibiotic function before the response of $\alpha\,\beta$ T cells (Hayday, 2000). Our results showed that the percentage of $\gamma\,\delta$ T cells decreased after transport (Fig. 2), likely resulting in the reduced function of this lymphocyte subset as mentioned above, therefore providing an opportunity for the invasion of pathogens, which may enhance the risk of infection by microorganisms under transport stress conditions.

Naïve Th cells differentiate into Th_1 or Th_2 cells after stimulation by antigens, providing cellular immune or humoral immune responses (Chaplin, 2010). IL-4 plays an important role in the differentiation of naïve Th cells toward Th_2 (Murphy, 1998); IL-6 can improve the early expression of IL-4 and inhibit the response of naïve Th cells to IFN-γ, which results in the differentiation of naïve Th cells into Th_2 (Dienz and Rincon, 2009). The decrease of percentage of Naïve Th cells (Fig. 2) with the increase of serum IL-4 and IL-6 (Fig. 3 A and B) indicates that naïve Th cells may have preferentially differentiated into Th_2.

CTL responds to stimulation by antigens by cell proliferation, production of IFN-γ and tumor necrosis factor (TNF)-α, thus killing target cells (Bassaganya-Riera *et al*., 2004; Laval *et al*., 2002; Wolfgang *et al*., 2003). The expression of CD8 on CTL cells would be decreased in the presence of IL-4 and absence of IFN-γ (Durrant and Metzger, 2010). The current study showed that the percentage of CTL decreased after transport (Fig. 2), which altered the immune status of transported animals, therefore increasing susceptibility to attack by pathogens.

The Th memory cell response to re-stimulation by antigen is cell proliferation, production of cytokines (IL-2, IFN-α, and IFN-γ), and promotion of B lymphocytes to release immunoglobulins (Charerntantanakul *et al*., 2006; Hontecillas and Bassaganya-Riera, 2003; Zuckermann and Husmann, 1996). The number of Th memory cells increases with age, likely because of consistent contact with foreign antigens. Moreover, porcine Th memory cells would be induced to proliferate once re-stimulated by antigens (Wasin and James, 2007).

The percentage of Th memory cells increased in the current study, likely due to the fact that transported pigs came into contact with antigens.

NK cells represent an important cell population of the innate immune system with the ability to spontaneously attack pathogen-infected and malignant body cells, as well as to produce immune-regulatory cytokines (especially IFN-γ), participating in the immune response (Wilhelm *et al*., 2009; Yokoyama, 2008). *In vitro* studies show that the activity of NK cells can be improved by IL-2 (Yata *et al*., 2009) and IFN-γ (Charley *et al*., 1985), but can be inhibited by IL-4 (Canning and Knoblock, 1992). Therefore, the increase of serum IFN-γ may have resulted in the percentage of NK cells being increased after transport in our experiment. However, the down regulation of IL-2 and IFN-γ mRNA, and the up regulation of IL-4 mRNA, suggest that the activity of NK cells might decrease as a consequence, and this may be the reason that transported animals are at high risk and susceptible to pathogens.

Different effects of transport stress on T helper cell cytokines

The balance between Th_1 and Th_2 is disrupted by stress, resulting in a new homeostasis (Ilia and Chrousos, 2002). Cytokines play an important role in the differentiation of naïve Th cells into Th_1 or Th_2 (Coffman, 2006). Our studies showed that serum concentrations of IFN-γ, IL-4, and IL-6 determined by ELISA increased post-transport (Fig. 4 A, B, and C), suggesting that transport stress induces naïve Th cells to differentiate into Th_1 or Th_2. The mRNA expression of cytokines was tested by semi-quantitative RT-PCR, showing that the expressions of cellular immune cytokines IFN-γ and IL-2 were down regulated, but humoral immune cytokines IL-4, IL-6, and IL-10 were up regulated (Fig. 5). Similar results have been found in a previous study (Qiongxia, 2011) suggesting that cellular immunity was enhanced in the early phase and then subsequently suppressed during transport, but humoral immunity continued to be strengthened. A previous study also showed that transport affects the humoral immune response of suckling calves by elevating the level of IgG_1 (Mackenzie *et al*., 1997), suggesting humoral immunity may play an important role in transport stress.

IL-6 plays an important role in promoting B lymphocyte differentiation into mature plasma cells, which release immunoglobulins, therefore enhancing humoral immunity (Commins *et al*., 2010). IL-6 also acts as a marker of bacterial infections in pigs (Caroline *et al*., 1998). In a previous study, the serum concentration of IL-6 increased after 1 h of transport, and the mRNA expression of IL-6 was up regulated and reached peak level after 4 h of transport (Qiongxia, 2011), which was similar to the findings in our experiment, indicating that bacterial infection might occur in transported animals and that humoral immunity was strengthened.

To summarize, stress response occurred on pigs during transport, which resulted in decreased leukocyte and lymphocyte numbers in the peripheral blood, as well as the percentages of γ δ T cells, naïve Th cells and CTL cells. However, the percentages of Th memory cells and NK cells increased after transport. Transport stress also changed serum concentrations of Th cell

cytokines and the mRNA expression of Th cell cytokines. These findings suggested that transport stress influences cellular immunity more than humoral immunity, and that humoral immunity might play an important in transport stress. Transported pigs could be at a high risk of attack by pathogens and proper measures should be taken before and after transport.

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REFERENCES

1. *Allen WM, Hebert CN, Smith LP,* 1974, Deaths during and after transportation of pigs in Great Britain, *Vet Rec*, 94, 212-4.
2. *Averós X, Knowles TG, Brown SN, Warriss PD, Gosálvez LF*, 2008, Factors affecting the mortality of pigs being transported to slaughter, *Vet Rec*, 163, 386-90.
3. *Bao E, Sultan KR, Bernhard N, Hartung J*, 2008, Expression and distribution of heat shock proteins in the heart of transported pigs, *Cell stress & chaperones* 13, 459-66.
4. *Bassaganya-Riera J, Thacker BJ, Yu S, Strait E, Wannemuehler MJ, Thacker EL*, 2004, Impact of immunizations with porcine reproductive and respiratory syndrome virus on lymphoproliferative recall responses of CD8+ T cells, *Viral Immunol* 17, 25-37.
5. *Bradshaw RH, Parrott RF, Forsling ML, Goode JAD, Lloyd M, Rodway RG et al.,* 1996, Stress and trevel sickness in pigs: effects of road transport on plasma concentrations of cortisol, beta-endorphin and lysine vasopressin, *Anim Sci*, 63, 507-16.
6. *Canning PC, Knoblock KF,* 1992, Modulation of *in vitro* porcine natural killer cell activity by recombinant interleukin-la, interleukin-2 and interleukin-4, *Immunology*, 76, 299-304.
7. *Carding SR, Egan PJ,* 2002, $\gamma\delta$ T cells: functional plasticity and heterogeneity, *Nat Rev Immunol* 2, 336-45.
8. *Caroline F, Wattrang E, Lisbeth F, Jensen KT, Wallgren P,* 1998, Evaluation of various cytokines, IL-6, IFN-α, IFN-γ, TNF-α) as markers for acute bacterial infection in swine - a possible role for serum interleukin-6, *Vet Immunol Immunopathol,* 64, 161-72.
9. *Chaplin DD,* 2010, Overview of the immune response, *J Aallergy Clin Immunol,* 125, S3-S23.
10. *Charerntantanakul W, Platt R, Johnson W, Roof M, Vaughn E, Roth JA,* 2006, Immune responses and protection by vaccine and various vaccine adjuvant candidates to virulent porcine reproductive and respiratory syndrome virus, *Vet Immunol Immunopathol,* 109, 99-115.
11. *Charley B, Petit E, Bonnardière CL,* 1985, Interferon-induced enhancement of newborn pig natural killing (NK) activity, *Ann Rech Vet,* 16, 399-402.
12. *Coffman RL*, 2006, Origins of the Th1-Th2 model: a personal perspective, *Nat Immunol,* 7, 539-41.
13. *Commins SP, Borish L, Steinke JW*, 2010, Immunologic messenger molecules: Cytokines, interferons, and chemokines, *J Allergy Clin Immun,* 125, S53-S72.
14. *Dalin AM, Magnusson U, Häggendal J, Nyberg L*, 1993, The effect of transport stress on plasma levels of catecholamines, cortisol, corticosteroid-binding globulin, blood cell count, and lymphocyte proliferation in pigs, *Acta Vet Scand,* 34, 59-68.

15. *Devilder MC, Allain S, Dousset C, Bonneville M, Scotet E*, 2009, Early triggering of exclusive IFN-γ responses of human Vγ9Vδ2 T cells by TLR-activated myeloid and plasmacytoid dendritic cells, *J Immunol*, 183, 3625-33.
16. *Deyi G, Bo H, Qinglan W, Shuxian J*, 1996, Effects of transport stress on plasma biochemistry in pigs, *Chi J Vet Sci,* 16, 285-9.
17. *Dienz O, Rincon M*, 2009, The effects of IL-6 on CD4 T cell responses, *Clin Immunol,* 130, 27-33.
18. *Durrant DM, Metzger DW*, 2010, IL-12 can alleviate Th17-mediated allergic lung inflammation through induction of pulmonary IL-10 expression, *Mucosal Immunol,* 3, 301-11.
19. *Earley B, Margaret M, Prendiville, DJ*, 2010, Effect of road transport for up to 24 hours followed by twenty-four hour recovery on live weight and physiological responses of bulls, *BMC Vet Res,* 6, 38.
20. *Frank B, Boyles SL, Riley JG*, 1984, Shipping suppresses lymphocyte blastogenic responses in Angus and Brahman Angus feeder calves, *J Anim Sci* 59, 576-83.
21. *Hayday AC*, 2000, γδ cells: a right time and a right place for a conserved third way of protection, *Annu Rev Immunol,* 18, 975-1026.
22. *Hong Y, Endong B, Ruqian Z, Qiongxia L*, 2007, Effect of transportation stress on heat shock protein 70 concentration and mRNA expression in heart and kidney tissues and serum enzyme activities and hormone concentrations of pigs, *Am J Vet Res* 68, 1145-50.
23. *Hontecillas R, Bassaganya-Riera J*, 2003, Differential requirements for proliferation of CD4+ and γδ+ T cells to spirochetal antigens, *Cellular Immunol,* 224, 38-46.
24. *Ilia JE, Chrousos GP*, 2002, Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity, *Ann N Y Acad Sci,* 966, 290-303.
25. *Jiefeng L, Yougang Z, Huanrong L, Nuowei Z, Wuren M, Guilin C et al.*, 2011, Enhancement of Astragalus polysaccharide on the immune responses in pigs inoculated with foot-and-mouth disease virus vaccine, *Int J Biol Macromol*, 49, 362-8.
26. *Laval F, Paillot R, Bollard S, Fischer L, Audonnet J-C, Andreoni C et al.*, 2002, Quantitative analysis of the antigenspecific IFN-γ T cell-mediated immune response in conventional outbred pigs, *Vet Immunol Immunopathol*, 90, 191-201.
27. *Mackenzie AM, Drennan M, Rowan TG, Dixon JB, Carter SD*, 1997, Effect of transportation and weaning on humoral immune responses of calves, *Res Vet Sci,* 63, 227-30.
28. *Meagher MW, Johnson RR, Young EE, Vichaya EG, Lunt S, Hardin EA et al.*, 2007, Interleukin-6 as a mechanism for the adverse effects of social stress on acute Theiler's virus infection, *Brain Behav Immun,* 21, 1083-95.
29. *Murphy KM*, 1998, T lymphocyte differentiation in the periphery, *Curr Opin Immunol,* 10, 226-32.
30. *Obernier JA, Baldwin RL*, 2006, Establishing an appropriate period of acclimatization following transportation of laboratory animals, *ILAR J,* 47, 364-9.
31. *Pérez MP, Palacio JM, Santolaria P, Aceña MC, Chacón G, Gascón M et al.*, 2002, Effect of transport time on welfare meat quality in pigs, *Meat Sci,* 61, 425-33.
32. *Pitard V, Roumanes D, Lafarge X, Couzi L, Garrigue I, Lafon ME et al.*, 2008, Long-term expansion of effector/memory V2-T cells is a specific blood signature of CMV infection, *Blood,* 112, 1317-24.
33. *Qiongxia L, Shuxia Z, Ruqian Z*, 2011, Transportation stress alters the expression of immunoregulatory cytokines in the porcine thymus, *Vet J* 187, 229-33.
34. *Salak-Johnson JL, McGlone JJ*, 2007, Making sense of apparently conflicting data: stress and immunity in swine and cattle, *J Anim Sci,* 85, E81-8.
35. *Stanger KJ, Ketheesan N, Parker AJ, Coleman CJ, Lazzaroni SM, Fitzpatrick LA*, 2005, The effect of transportation on the immune status of Bos indicus steers, *J Anim Sci,* 83, 2632-6.
36. *Takamatsu HH, Denyer MS, Stirling C, Cox S, Aggarwal N, Dash P et al.*, 2006, Porcine γδ T cells: possible roles on the innate and adaptive immune responses following virus infection, *Vet Immunol Immunopathol,* 112, 49-61.
37. *Takamatsu HH, Denyer MS, Wileman TE*, 2002, A sub-population of circulating porcine γδ T cells can act as professional antigen presenting cells, *Vet Immunol Immunopathol*, 87, 223-4.
38. *Tian W, Yunfei G, Eileen S, Todd DC, John FA, Welte T et al.*, 2006, γδ T cells facilitate adaptive immunity against West Nile virus infection in mice, *J Immunol,* 177, 1825-32.

39. *Von Borell EH*, 2001, The biology of stress and its application to livestock housing and transportation assessment, *J Anim Sci*, 79, E260-7.
40. *Warriss PD, Bevis EA, Ekins PJ*, 1989, The relationships between glycogen stores and muscle ultimate pH in commercially slaughtered pigs, *Br Vet J,* 145, 378-83.
41. *Wasin C, James AR*, 2007, Biology of porcine T lymphocytes, *Anim Health Res Rev*, 7, 81-96.
42. *Wilhelm G, Kaser T, Saalmuller A*, 2009, Porcine T lymphocytes and NK cells – an update, *Dev Comp Immunol,* 33, 310-20.
43. *Wolfgang S, Duvigneau C, Pietschmann P, Höller K, Hartl R, Wahl K et al.*, 2003, Parameters of humoral and cellular immunity following vaccination of pigs with a European modified-live strain of porcine reproductive and respiratory syndrome virus (PRRSV), *Viral Immunol,* 16, 335-46.
44. *Yata A, Nakabayashi K, Wakahashi S, Maruo N, Ohara N, Maruo T*, 2009, Suppression of progesterone production by stresscopin/urocortin 3 in cultured human granulosa-lutein cells, *Hum Reprod,* 24, 1748-53.
45. *Yokoyama WM*, 2008, Mistaken notions about natural killer cells, *Nat Immunol,* 9, 481-5.
46. *Yoshioka G, Imaeda N, Torimoto Y, Ohtani T, Hayashi K*, 2004, Influence of transport stress on serum cortisol and thyroid hormones in pigs with Halothane gene, *J Anim Sci*, 75, 451-6.
47. *Zuckermann FA, Husmann RJ*, 1996, Functional and phenotypic analysis of porcine peripheral blood CD4/CD8 double-positive T cells, *Immunology,* 87, 500-12.

UTICAJ STRESA TOKOM TRANSPORTA SVINJA NA SUBPUPULACIJE PERIFERNIH LIMFOCITA I Th CITOKINE

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SADRŽAJ

U cilju ispitivanja uticaja stresa tokom transporta na supopulacije limfocita i Th citokina, uzorci krvi su prikupljani od svinja pre i posle transporta. Osim toga, određivana je i aktivnost enzima kreatin kinaze (CK), alkalne fosfataze (ALP), laktat dehidrogenaze (LDH) kao i koncentracija glukoze i kortizola. U studiji je određivan broj leukocita i limfocita, procenat različitih subpopulacija limfocita, koncentracija Th citokina i ekspresija njihovih mRNK. Posle transporta, aktivnost CK kao i koncentracija IL-4 i glukoze su bili značajno povećani ($p<0,01$). Aktivnost LDH, procenat Th celija pamćenja i NK celija i koncentracija IFN γ bili su takođe značajno povećani ($p<0,05$). Koncentracija kortizola i broj leukocita i limfocita bili su značajno smanjeni ($p<0,01$) kao i procenat $\gamma\delta$ T celija, naivnih Th celija i citotoksičnih T limfocita ($p<0,05$). Ekspresija mRNK za IL-2 i IFN γ bila je takođe značajno smanjena ($p<0,01$ i $p<0,05$ respektivno). To nije bio slučaj sa IL-4, IL-6 i IL-10, ali je samo u slučaju IL-10 utvrđena visoko statistički značajna razlika ($p<0,01$). Ovi rezultati ukazuju da stres tokom transporta može da dovede do imunosupresije koja se u većoj meri odražava na celularni odgovor. Može se očekivati, da posle transporta, bude povećana i osetljivost na infekcije i potrebno je preduzimati mere da se ovo izbegne.