Journal of Animal and Veterinary Advances 9 (21): 2748-2751, 2010
ISSN: 1680-5593

Investigation and Comparative Study on Haematological Traits, Lysozyme Concentration and T Lymphocyte Subpopulation in Three Pig Breeds

[1]Y. Liu, [1]Y.R. Luo, [1]X. Lu, [1]X.T. Qiu, [1]W.X. Fu, [1]J.P. Zhou,
[1]X.Y. Liu, [1]Q. Zhang and [2]Z.J. Yin
[1]Key Laboratory of Animal Genetics and Breeding of the Ministry of Agriculture,
College of Animal Science and Technology, China Agricultural University,
Beijing 100193, P.R. China
[2]College of Animal Science and Technology, Anhui Agricultural University,
Hefei 230036, P.R. China

Abstract: In this study, haematological traits, lysozyme concentration and T lymphocyte subpopulation as markers of innate immunity were detected and compared among Landrace, Large White and a chinese indigenous breed, Songliao Black pig. The animals were of the same age and kept under the same environmental conditions to reduce non-genetic variation in immune traits; they were all apparently healthy and were vaccinated by CSF live vaccine at 21 days of age. Except LY%, MO%, $CD4^-CD8^-$% and RDW, the other detected immune traits were significant difference between before (20 days) and after vaccination (35 day). While the values of MCV, MCH, MCHC, MO%, $CD4^+CD8^+$% and $CD4^+CD8^+/CD4^-CD8^+$ values decreased after vaccination, the others increased. The values of WBC, GR, LY, MO, $CD4^+CD8^-$% and $CD4^+CD8^+$% were significant difference among Large White, Landrace and Songliao Black pig ($p<0.05$). Our analysis confirms that Songliao Black pig has better innate immune level than Landrace and Large White. The animal resource population was suggested to be appropriate to investigate further the QTL and genes contributing to differences on these innate immune traits in pigs.

Key words: Pig, haematological traits, lysozyme, T lymphocyte, Comparative analysis, non genetic variation

INTRODUCTION

Animal disease resistance is the result of interaction of innate (non-specific) and specific immunity and both of them are under genetic control (Muller and Brem, 1991). The innate immune system comprises the cells and mechanisms that defend the host from infection by other organisms, in a non-specific manner and is general resistance to disease; it reflects the overall defense capacity against diseases (Alberts *et al*., 2002). The improvement of the general resistance to disease for enhancing the overall immune function and identification of these variants of immune traits may be suitable for the genetic improvement through selection (Lamont, 1998). However, innate immune traits and disease resistance also impact upon animal performance. Genetically improving disease resistance in pigs may or may not impinge on performance, depending upon the pathogen and the nature of the infectious challenge (Colditz, 2002). Chinese breed Meishan pig and western breed Large Whites pig have been appeared to differ in resistance to a wide variety of pathogens and also differ in various innate immune traits (Clapperton *et al*., 2005; Sutherland *et al*., 2005). Genetic component to variation in several innate immune traits has been reported in pigs (Edfors-Lilja *et al*., 1994; Clapperton *et al*., 2005) and some Quantitative Trait Llocus (QTLs) also has been identified in pigs (Edfors-Lilja *et al*., 1998; Gong *et al*., 2010; Reiner *et al*., 2007; Wattrang *et al*., 2005).

Haematological traits are essential parameters for evaluating the health status of individual animals and herds (Gong *et al*., 2010). Lysozyme concentration in tissue fluids also reflect the number of activated macrophages (Hyyppa *et al*., 1989). T-lymphocytes represent an important cell population of the immune system and perform a wide array of functions in immune regulation, inflammation and protective immune responses (Summerfield *et al*., 1996). Identifying the variants in these innate immune traits would be a helpful step towards exploring any link to disease resistance capacity and improve a measure to advance general health in the pig. In order to investigate the differences of innate immune traits

Corresponding Author: Yin Zongjun, Department of Animal Science, College of Animal Science and Technology, Anhui Agricultural University, Hefei 230036, China

on different pig breeds and further build an animal resource population for QTL mapping studies, we firstly chose and detected the haematological traits, lysozyme concentration and T lymphocyte subpopulation which represent different aspects of innate immunity. At last, it also further compared the haematological traits, lysozyme concentration and T lymphocyte subpopulation before and after vaccination among Landrace, Large White and a Chinese indigenous breed, Songliao Black pig.

MATERIALS AND METHODS

Animals: The animals consisted of 338 piglets distributed in three pig breeds including Large White (169), Landrace (84) and Songliao Black pig (85). All pigs were raised under the standard indoor condition in 2007 and 2008 at the experimental farm of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China. All pigs were apparently healthy and vaccinated with Classical Swine Fever (CSF) live vaccine at 21 days of age.

Blood samples: Two measurements were performed on 20 days old and 35 days old piglets respectively. For each piglet, 10 mL blood was collected via the external jugular vein. The first blood samples were collected from each piglet one day before the vaccination (20 days) and two weeks after the vaccination; the second blood samples were collected (35 days). All blood samples were divided into two tubes: the first tube contained EDTA anti-coagulant for the measurement of haematological traits and T lymphocyte subpopulation; the second tube was directly injected into VACUETTE® Serum Clot Activator tubes for detection for lysozyme concentration.

Measurement of haematological traits and lysozyme concentration in blood: Haematological traits which consist of mainly three components, including leukocyte traits, erythrocyte traits and platelet traits were measured. Total 18 blood parameters include 7 leukocyte traits (White Blood Cell Count (WBC), neutrophilic granulocyte count (GRAN) and percentage (GR%), lymphocyte count (LYMF) and percentage (LY%), monocytes count (MONO) and percentage (MO%)), 7 erythrocyte traits (Red Blood Cell (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Red Blood Cell volume distribution width (RDW)), 4 platelet traits (blood Platelet counts (PLT), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Plateletocrit (PCT). All these blood routine parameters were measured by MEK-6318K type full automatic Hematology Analyzer (Nihon Kohden, Japan). Lysozyme Concentration (LSZ) in serum was measured by turbidimetric analysis using the lysozyme testing kit (Jiancheng Institute of Biotechnology, Nanjing, China).

Measurement of T lymphocyte subpopulation in blood: T lymphocyte subpopulations ($CD4^+CD8^+$%, $CD4^+CD8^-$%, $CD4^-CD8^+$% and $CD4^-CD8^-$%) were detected by EPICS Flow Cytometer (Beckman-Coulter Company, USA) with an argon laser with an excitation wavelength of 488 nm and using FITC-CD4/PE-CD8 monoclonal antibody (Serotech Ltd., England). The percentage of monoclonal antibody CD8/CD4 cells was obtained for each sample tested and the purity of the separated fractions was ≥98%.

RESULTS AND DISCUSSION

Except LY%, MO% and RDW, the other detected haematological traits were significant difference between before and after vaccination by paired t-test (Table 1). The values of MCV, MCH, MCHC and MO% decreased and

Table 1: Comparative analysis of haematological traits, T lymphocyte subpopulations, lysozyme concentration before and after vaccination

Traits[1]	Before vaccination (Mean±SE)	After vaccination (Mean±SE)	p-value
WBC ($g\ L^{-1}$)	12.24±0.31	18.49±0.39	<0.0001
GRAN ($g\ L^{-1}$)	2.81±0.14	4.83±0.27	<0.0001
GR (%)	22.18±0.80	24.00±0.91	0.0400
LYMF ($g\ L^{-1}$)	7.46±0.20	10.86±0.22	<0.0001
LY (%)	61.98±0.79	60.77±0.82	0.1400
MONO ($g\ L^{-1}$)	1.98±0.07	2.77±0.08	<0.0001
MO (%)	15.83±0.37	15.27±0.32	0.1700
RBC ($g\ L^{-1}$)	5.55±0.06	6.16±0.07	<0.0001
HGB ($g\ L^{-1}$)	113.42±1.14	118.49±1.36	<0.0001
HCT (%)	34.96±0.36	37.09±0.42	<0.0001
MCV (fL)	62.95±0.27	57.73±0.24	<0.0001
MCH (pg)	20.54±0.11	18.44±0.07	<0.0001
MCHC ($g\ L^{-1}$)	326.12±1.24	320.37±1.15	<0.0001
RDW (%)	17.62±0.17	17.74±0.18	0.6900
PLT ($g\ L^{-1}$)	395.24±9.34	462.4±10.69	<0.0001
MPV (fL)	9.69±0.08	9.09±0.08	<0.0001
PDW (%)	14.98±0.07	14.72±0.07	<0.0001
PCT (%)	0.37±0.01	0.4±0.010	0.0100
$CD4^+CD8^+$ (%)	8.63±0.23	10.38±0.27	<0.0001
$CD4^+CD8^-$ (%)	19.22±0.44	14.19±0.39	<0.0001
$CD4^-CD8^+$ (%)	37.73±0.80	40.41±0.62	0.0014
$CD4^-CD8^-$ (%)	34.40±0.63	35.27±0.63	0.3165
LSZ ($\mu g\ L^{-1}$)	14.51±5.67	16.96±6.67	0.0017

[1]WBC, White Blood Cell count; GRAN, neutrophilic granulocyte count; GR% neutrophilic granulocyte count percentage; LYMF, lymphocyte count; LY% lymphocyte count percentage; MONO, Monocytes count; MO%, percentage; RBC, Red Blood Cell; HGB, Hemoglobin; HCT, Hematocrit; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; RDW, Red blood cell volume distribution width; PLT, blood platelet counts; MPV, Mean Platelet Volume; PDW, Platelet Distribution Width; PCT, Plateletocrit; $CD4^+CD4^-CD8^+CD8^-$ T lymphocyte subpopulation; LSZ, Lysozyme concentration

those of the other haematological traits increased after vaccination. The values of WBC, GR%, LY and MO were significant difference among Large White, Landrace and Songliao Black pig (Table 2). The breed and age of pig are important factors influencing immune response to various infectious challenges (Sutherland *et al*., 2005). In a study for two Australian pig breeds, WBC and $CD4^+$. T lymphocyte count of Large White are higher than Duroc after leptospira vaccination (Nguyen *et al*., 1998). Moreover, compared with the Large White, Meishan pig has higher neutrophils and monocyte counts and lower lymphocyte counts (Clapperton *et al*., 2005). In this study, WBC, PLT, GR% and GRAN of Chinese indigenous breed Songliao Black pig were higher increased after vaccinated with CSF live vaccine than western pig breed Landrace and Large White, this is similar to Meishan pig.

Lysozyme, also called bacteriolysin is found in saliva and has been shown to have an antibody-independent defense function (Hyyppa *et al*., 1989). Lysozyme concentration in serum was significant difference between 20th and 35th day ($p = 0.0017$) and the value of LSZ was also increased after vaccination (Table 1). Among three pig breeds, the value of LSZ was significant difference ($p<0.05$) and Songliao Black pig has higher level than Landrace and Large White (Table 2). Lysozyme was synthesized and released by macrophages, the elevation in lysozyme levels consequent upon the growth of antigenic tumors may be a reflection of an increase in the number of macrophages (Currie and Eccles, 1976). The results suggest that Songliao Black pig have much more macrophages numbers than landrace, Large White. T lymphocytes represent an important cell population of the immune system. The detection of T lymphocyte subpopulation by Flow Cytometer was shown in Fig. 1. Three of four T lymphocytes subpopulation except $CD4^-CD8^-$ were very significantly different before and after vaccination ($p<0.01$) and the percentage of $CD4^+CD8^+$, $CD4^-CD8^+$ and $CD4^-CD8^-$ T lymphocyte increased (Table 1).

In addition, the percentage of three T lymphocytes except $CD4^-CD8^+$ was also significantly different among three pig breeds (Table 2). The variation in the percentage of $CD4^+CD8^+$ T lymphocyte is confirmed increasing with the age of the animals (Summerfield *et al*., 1996). The extrathymic $CD4^+CD8^+$ T lymphocyte subpopulation of swine contains MHC class II restricted antigen-specific memory T helper cells and this $CD4^+CD8^+$ T lymphocyte subpopulation could be characterized as mature T lymphocytes morphologically and phenotypically (Pescrovitz *et al*., 1994). Lohse *et al*. (2006) also reported that increase of $CD4^+CD8^+$ lymphocyte may contribute to host resistance against infectious diseases. Therefore, $CD4^+CD8^+$ T lymphocyte is an important reflection of immune status in pigs. In this study, the percentage of $CD4^+CD8^+$ in Songliao Black pig has higher level than Large White but is lower level than that of Landrace (Table 2). The results suggested Songliao Black pig have better immune capacity in three pig breeds.

In this experiment, various innate immune parameters detected differences among three pig breeds and all parameters belong to quantitative traits. Significant differences of immune traits before and after vaccination

Table 2: Multiple comparative analysis for difference value of haematological traits, lymphocyte subpopulations and lysozyme concentration in three pig breeds

Traits	Landrace (n = 84)	Large white (n = 169)	Songliao black (n = 85)
WBC (g L^{-1})	4.41±0.910^a	6.22±0.560^{ab}	7.66±1.120^b
GRAN (g L^{-1})	1.57±0.520^a	1.27±0.290^a	3.96±0.800^b
GR (%)	2.29±2.090^a	-0.69±1.240^a	7.53±2.440^b
LYMF (g L^{-1})	2.43±0.550^a	3.86±0.340^b	3.07±0.630^{ab}
LY (%)	-1.47±2.220^{ab}	0.57±1.920^a	-5.45±2.310^b
MONO (g L^{-1})	0.40±0.230^a	1.04±0.120^b	0.63±0.220^{ab}
MO (%)	-0.76±1.140	0.14±0.530	-1.96±0.890
RBC (g L^{-1})	0.78±0.190	0.64±0.120	0.44±0.150
HGB (g L^{-1})	0.68±3.400^a	9.26±2.310^b	2.08±2.840^{ab}
HCT (%)	2.20±1.030	3.17±0.710	0.74±0.820
MCV (fL)	-4.50±0.440	-5.51±0.340	-4.85±0.340
MCH (pg)	-2.88±0.340^a	-2.08±0.140^b	-1.51±0.140^b
MCHC (g L^{-1})	-22.76±4.550^a	-3.55±2.210^b	1.50±1.810^b
RDW (%)	-0.12±0.330	0.07±0.180	0.16±0.220
PLT (g L^{-1})	49.42±27.55	68.65±19.46	83.22±21.49
MPV (fL)	-0.35±0.190	-0.66±0.100	-0.74±0.120
PDW (%)	-0.17±0.130	-0.32±0.100	-0.12±0.120
PCT (%)	0.02±0.030	0.03±0.020	0.04±0.020
$CD4^+CD8^+$ (%)	0.06±0.010^a	0.01±0.010^b	0.03±0.010^{ab}
$CD4^+CD8^-$ (%)	-0.05±0.010^a	-0.09±0.010^b	-0.06±0.000^{ab}
$CD4^-CD8^+$ (%)	0.03±0.020	0.05±0.010	0.00±0.020
$CD4^-CD8^-$ (%)	-0.03±0.010^b	0.01±0.010^a	0.03±0.010^a
LSZ ((μg L^{-1}))	13.31±1.560^a	15.99±1.150^{ab}	16.92±1.140^b

^{a,b}Signed by small letters differ significantly at $p<0.05$

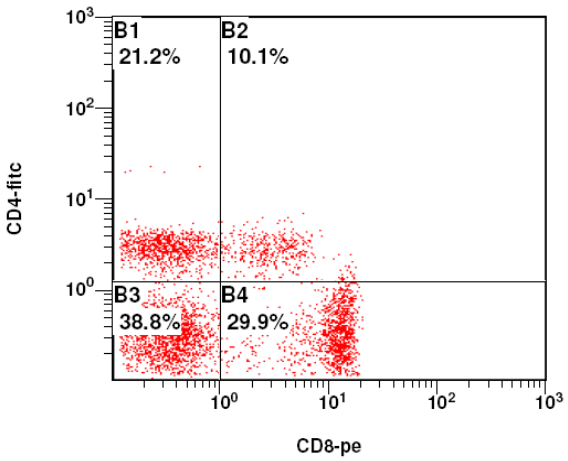

Fig. 1: Alloantigen-specific response of porcine T lymphocyte subpopulations and CD8 versus CD4 expression of the un-separated T lymphocyte was detected by Flow Cytometer. The fluorescence intensities of CD8 (PE, abscissa) and CD4 (FITC, ordinate) are displayed in two-dimensional contour plot

reflect whether immune responses or the strength of its levels are induced. The compared results may indicate Songliao Black pig has higher immune capacity or disease resistance capacity than other two pig breeds.

Furthermore, the detected results also suggested the pig resource populations can be used for QTL mapping studies to detect genome regions containing genes contributing to differences of these immune traits in the pig.

CONCLUSION

We detected differences among Landrace, Large White and Songliao Black pigs in several innate immune traits before and after vaccination. Differences in the measured immune traits could be because the breeds differ in their basal levels of these traits and in their immune capacity. The results indicated the Chinese indigenous breed, Songliao Black pig, may has higher immune capacity or disease resistance than Landrace, Large White. These results also suggested genetic differences in these marker traits were provided evidence for disease resistance capacity in different pig breeds and the resource populations may be suitable for QTL mapping studies to detect genome regions containing genes contributing to differences in these immune traits.

ACKNOWLEDGEMENTS

This research is supported by China Postdoctoral Science Foundation Funded Project (Grand No. 20100470412); National Key Project for Basic Research and Development Plans (Grant No. 2006CB102104) and the Project of Anhui Scientific Technology Plan (Grant No. 07010300136).

REFERENCES

Alberts, B., A. Johnson, J. Lewis, M. Raff, K. Roberts and P. Walter, 2002. Molecular Biology of the Cell. 4th Edn., Garland Science, New York, pp: 1027-1060.

Clapperton, M., S.C. Bishop and E.J. Glass 2005. Innate immune traits differ between meishan and large white pigs. Vet. Immunol. Immunopathol., 104: 131-144.

Colditz, I.G., 2002. Effects of the immune system on metabolism: Implications for production and disease resistance in livestock. Livest. Product. Sci., 75: 257-268.

Currie, G.A. and S.A. Eccles, 1976. Serum lysozyme as a marker of host resistance. Br. J. Cancer, 33: 51-59.

Edfors-Lilja, I., E. Wattrang, L. Marklund, M. Moller, L.A. Eklund, L. Andersson and C. Fossum, 1998. Mapping quantitative trait loci for immune capacity in the pig. J. Immunol., 160: 829-835.

Edfors-Lilja, I., E. Wattrang, U. Magnusson and C. Fossum, 1994. Genetic variation in parameters reflecting immune competence of swine. Vet. Immunol. Immunopathol., 40: 1-16.

Gong, Y.F., X. Lu, Z.P. Wang, F. Hu and Y.R. Luo *et al*., 2010. Detection of quantitative trait loci affecting haematological traits in swine via genome scanning. BMC Genet., 11: 56-56.

Hyyppa, T., L. Karhuvaara, J. Tenovuo, M. Lumikari and P. Vilja, 1989. Antimicrobial factors in whole saliva of human infants: A longitudinal study. Pediatr. Dent., 11: 30-36.

Lamont, S.J., 1998. Impact of genetics on disease resistance. Poult. Sci., 77: 1111-1118.

Lohse, L., J. Nielsen and L. Eriksen, 2006. Long-term treatment of pigs with low doses of monoclonal antibodies against porcine CD4 and CD8 antigens. APMIS., 114: 23-31.

Muller, M. and G. Brem, 1991. Disease resistance in farm animals. Experientia, 47: 923-934.

Nguyen, V.P., C.W. Wong, G.N. Hinch, D. Singh and I.G. Colditz, 1998. Variation in the immune status of two Australian pig breeds. Aust. Vet. J., 76: 613-617.

Pescrovitz, M.D., B. Aasted, A. Canals, J. Dominguez and J.S. Vizcaino *et al*., 1994. Analysis of monoclonal antibodies reactive with the porcine CD2 antigen. Vet. Immunol. Immunopathol., 43: 229-232.

Reiner, G., R. Fischer, S. Hepp, T. Berge, F. Kcehler and H. Willems, 2007. Quantitative trait loci for red blood cell numbers in swine. Anim. Genet., 38: 447-452.

Summerfield, A., H.J. Rziha and A. Saalmuller, 1996. Functional characterization of porcine $CD^{4+}CD^{8+}$ extrathymic Tlymphocytes. Cell. Immunol., 168: 291-296.

Sutherland, M.A., S.L. Rodriguez-Zas, M. Ellis and J.L. Salak-Johnson, 2005. Breed and age affect baseline immune traits, cortisol and performance in growing pigs. J. Anim. Sci., 83: 2087-2095.

Wattrang, E., M. Almavist, A. Johansson, C. Fossum and P. Wallgren *et al*., 2005. Conformation of QTL on porcine chromosome 1 and 8 influencing Leukocyte numbers, haematological parameters and leukocyte function. Anim. Genet., 36: 337-345.