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**GENETIC VARIATION IN IMMUNE  
COMPETENCE OF PIGS IN VIETNAM AND  
AUSTRALIA**

by

NGUYEN VAN PHUC

A thesis submitted for the degree of Master of Rural Science in the Department of  
Animal Science, School of Rural Science,  
University of New England, Armidale, NSW 2351, Australia

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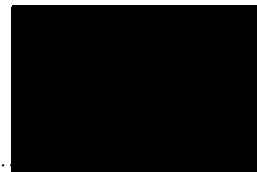
## PREFACE

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*The studies presented in this thesis are original, and were completed by the author, a postgraduate student in the Department of Animal Science, School of Rural Science, Faculty of Sciences, The University of New England, Armidale, NSW 2351, Australia, under the supervision of Dr Geoff Hinch and Dr William Wong.*

*I certify that the substance of this thesis has not already been submitted for any other degree and is not currently being submitted for any other degree.*

*I certify that to the best of my knowledge any help received in preparing this thesis, and all the sources used, have been properly acknowledged in this thesis.*

A solid black rectangular box used to redact the author's signature.

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Nguyen Van Phuc

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## Abbreviations

Ab	: Antibody
ConA	: Concanavalin A
cpm	: Count per minute
DNA	: Deoxyribonucleic acid
DNCB	: Dinitrochlorobenzene
DTH	: Delayed-type hypersensitivity
ELISA	: Enzyme-link immunosorbent assay
FACS	: Fluorescence activated cell sorter
g	: gram
HEW	: Hen egg white lysozyme
KLH	: Keyhole limpet haemocyanin
LST	: Lymphocyte stimulation test
mAb	: Monoclonal antibody
MAT	: Microscopic agglutination test
MHC	: Major histocompatibility complex
ml	: millilitre
mg	: milligram
PHA	: Phytohaemagglutinin
PCV	: Pack cell volume
RBC	: Red blood cell
SCC	: Somatic cell counts
SEM	: Standard error
SI	: Stimulation index
SLA	: Swine lymphocyte antigen
WBC	: White blood cell
$\mu$ l	: microlitre
$\mu$ g	: microgram

## ABSTRACT

. The overall aim of the studies presented in this thesis was to examine the variation in immune competence of different pig breeds commonly raised in Vietnam and Australia.

In the first study, three of the most popular pig breeds in Vietnam: Mong Cai (indigenous), Large White and Landrace (exotic breeds to Vietnam) were used. A total of 105 weaner piglets of both sexes, aged 8 weeks, from 24 litters were studied. The piglets were the offspring of 15 sires. Under the same environmental and feeding conditions, both humoral and cellular immune responses were tested in these piglets. Antibody responses to injections of keyhole limpet haemocyanin (KLH) and ovalbumin after 7, 14, 21, 28, 35 and 42 days were measured using ELISA. Skin reactivity to mitogens was induced by intradermal injections of phytohaemagglutinine (PHA) and dinitrochlorobenzene (DNCB) at 10 weeks of age and assessed by measuring the change in skin thickness. Total and differential white blood cell (WBC) counts were also measured at this time.

The peak antibody titres ( $\log_2$ ) to KLH were achieved at 14 days after the first immunisation. The titres were  $12.5 \pm 1.18$ ;  $8.7 \pm 0.83$  and  $9.6 \pm 0.78$  for Mong Cai, Landrace and Yorkshire, respectively. There appeared to have significant differences between breeds in the peak antibody response ( $P < 0.001$ ) where Mong Cai pigs had a higher titre level. Skin thickness was measured at 0, 24,

48 and 72 hours after immunisation with a maximum thickness measured appearing at 24 h. The Mong Cai breed significantly showed the highest reactivity to DNCB ( $P < 0.05$ ) (1.04 mm, a 45.6% increase) of the three breeds tested while the skin reactivity of Landrace (0.86 mm, a 29.2% increase) was stronger than that of Yorkshire (0.74 mm a 23.3% increase). The number of leucocytes in the blood of the piglets was found to be significantly different ( $P < 0.05$ ) between the three breeds at their ages of 10 weeks. Total WBC counts were  $23.03 \pm 0.8$ ,  $19.43 \pm 0.9$  and  $20.12 \pm 0.6$  for Mong Cai, Landrace and Yorkshire, respectively. Again, Mong Cai pigs showed a higher leucocyte count than the others.

In the second study which was carried out in the Department of Primary Industry in Brisbane, Australia a total of 48 piglets from two local breeds; Large White and Duroc were compared. Again, all animals were kept under the same environmental and feeding conditions. All pigs were immunised against porcine leptospirosis. The leucocyte counts were measured at different time points before and after the primary and secondary immunisation. Large White piglets consistently showed significantly higher total leucocyte counts than the Duroc piglets ( $P < 0.05$ ). There was a significant decrease ( $P < 0.05$ ) in lymphocytes : neutrophils proportion for each breed after the immunisations, but no significant difference in the lymphocyte counts between the two breeds was observed. However, the distribution of neutrophils ( $P < 0.01$  or  $P < 0.05$ ) was significantly higher in Duroc after each of the two immunisations, whereas

Large White pigs had higher eosinophil counts ( $P < 0.05$ ) at the week after the first immunisation.

Lymphocyte stimulated test (LST) was used in the study to test variation in the cell-mediated response. Interestingly, there was a significant difference ( $P < 0.05$ ) between the two breeds before immunisation where the Large White breed showed higher lymphocyte responsiveness to the stimulation of T cell mitogen, namely concanavalin A (Con A), than Duroc pigs, but neither sire nor sex of these breeds caused any significant variations in the Con A-induced lymphocyte response. Variation in blood lymphocyte phenotypes was also examined using fluorescence activated cell sorter (FACS) analysis. Significant increases ( $P < 0.05$ ) in all phenotypes within each of the two breeds were noticed in response to each of the two immunisations. In particular, the Large White breed showed significantly higher numbers of  $CD2^+$  and  $CD4^+$  cells ( $P < 0.01$  and  $P < 0.05$  respectively) than Duroc after each of the two immunisations. Of interest, there were also some significant sire effects ( $P < 0.05$ ) on  $CD8^+$  cells within the Large White breed at 7 days after the first immunisation, but no sex effect on any phenotypes of lymphocytes was observed between or within breeds.

In serology, variation in the levels of serum IgG was examined using a direct ELISA method. A significant increase ( $P < 0.05$ ) after the immunisations within each breed was noticed. However, no significant differences between breeds or sexes were detectable although there were sire differences within each breed



before the initial vaccination ( $P < 0.05$ ). The antibody response to vaccination against leptospirosis was measured at the WHO-FAO Leptospirosis Reference Laboratory, Brisbane using a standard method called microscopic agglutination test (MAT). No significant variations in antibody titres were found in terms of effects exerted by breeds, sires and sexes.

The results have clearly demonstrated significant differences in the immune competence within breeds of pigs in both Vietnam and Australia and has demonstrated potential breed differences although population sizes was too small to be certain. The large difference in immune responsiveness of breeds and sires indicates that further studies of the heritability of immune function and are needed and that further studies are warranted. In particular, the indigenous breed which constantly appeared to be more immunologically responsive than the exotic breeds in Vietnam would be very valuable in future study and breeding program for disease resistant pigs.

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