Chapter 3

VARIATION IN THE IMMUNE RESPONSIVENESS OF THREE PIG BREEDS IN VIETNAM

3.1. INTRODUCTION

There a number of genetic and non-genetic factors which have direct or indirect influence on the immune system of animals (Siegel and Gross, 1980). Although most studies of genetic variation in antigen recognition and antibody production have focussed on specific loci, especially with the aid of inbred populations, experiments based on selecting high and low immune responsiveness to non-specific challenge or particular diseases have also been used to assess genetic variation in immune responses and their usefulness in livestock industries.

Genetic variation in immune responsiveness has been widely reported in many different species of domestic animals. Resistance to diseases is believed to be related to general immune responsiveness which should be considered as one of the components of the selection criteria used in selecting breeding animals (Edfors-Lilja et al, 1994). If immunological parameters are to serve as a basis for genetic selection, it is necessary to determine genetic variation in the parameters (Buschmann, 1982).

In pigs, Buschman et al (1974) have long initiated the idea of investigating into the use of *in vivo* immunological parameters as a basis for selection for disease resistance. The most intensively studied immune response traits in pigs have been those being able to reflect both the humoral and cell-mediated immunity, although some studies have also focused on the innate immunity (Mallard et al, 1991; Edfors-Lilja et al, 1991,1994). In this regard, moderate to high heritability have been estimated for several immunological traits, for example, white blood cell counts and polymorphonuclear leucocyte (PMN) counts, antibody responses, phagocytic capacity of PMNs (Edfors-Lilja et all, 1994). Within and between breed variations in immune responses of pigs to a variety of antigens and diseases have been demonstrated (Rothschild, 1985, 1990). For example, genetic variation in specific antibody responses to both simple and complex antigens have been well documented (Buschmann et al, 1974; Edfors-Lilja et al, 1985; Rothschild et al, 1984a,b,c,d; Mallard et al, 1989). Genetic differences in non-specific serum immunoglobulin levels and in cell-mediated response measured *in vitro* (Mallard et al, 1989 ; Edfors-Lilja et al 1989; Jensen and Christensen 1981) were also reported in pigs. Breeds difference in the killing capacity of porcine monocytes have recently been demonstrated by Buschman et al (1980).

The present study was designed to study differences in immune responsiveness of three pig breeds in Vietnam. They were indigenous Mong Cai breed and Landrace and Yorkshire, which were two exotic breeds commonly raised in the

coastal region of North Vietnam. Mong Cai breed is a native breed largely distributed in the Northern part of Vietnam. A brief historic and economic background of these three breeds is included as follows:

Mong Cai pigs account for more than 25 % of indigenous pigs according to Statistic Department, State Plan Committee, Vietnam (1993). They are bred in all the provinces of the Red River bank and the coastal areas. These pigs are well known not only for their prolificacy (12 ± 0.6 piglets/litter) but also for their good care especially for the offspring (survival at weaning 80-85%) (Doanh et al, 1984). They are well adapted to the harsh environment and have been reported to be relatively resistant to diseases in comparison with other pig breeds living in the same region (Giang et al, 1973). Because of their valuable characteristics Mong Cai pigs have been playing a significant role in the Genetic Resources Conservation Programme in Vietnam. In particular, a large number of crossbreeding programs have been based on Mong Cai pigs using the female line with a view to improving the growth performance and taking advantage of the heterosis from maternal traits.

Landrace and Yorkshire animals are two of the earliest exotic pig breeds imported to Vietnam. So far they have adapted well to the high-humidity tropical climate and are widely distributed throughout Vietnam from North to South. The Yorkshire pigs are in fact considered to be the principal breed in Vietnam, and their populations have increased quickly through pure-breed multiplication (Hai et al, 1995). Since 1990, with the government's

encouragement to produce exportable pork, high level of lean meat production has been required, and these two exotic breeds have best met this demand. As a result, Landrace and Yorkshire pigs have also become the most popular breeds raised in the State farms as well as by small private holders. Landrace and Yorkshire boars are now used for crossbreeding programs with local breeds to improve the productivity in terms of lean meat yield.

3.2 MATERIALS AND METHODS

3.2.1 Animals

The piglets of three pure breeds used in this study were obtained from the North of Vietnam, namely Mong Cai, Landrace and Yorkshire.

A total of 106 weaner piglets, aged 8 weeks, from 24 litters were studied. The piglets were of both sexes. Fifteen boars were used. Eight boars sired one litter each, 5 boars 2 litters each and 2 boars 3 litters. A total of 35 piglets of the Mong Cai breed which were purchased from Dong Trieu piggery, Hanoi., Vietnam, arose from 9 litters (5 boars and 9 sows). A total of 35 piglets of the Landrace breed, which was raised in the Pig Research Centre, National Institute of Animal Husbandry, Hanoi, arose from 8 litters (6 boars and 8 sows). A total of 36 piglets of the Yorkshire breed which was also raised in the Pig Research Centre, arose from 7 litters (4 boars and 7 sows),

The experiment was conducted at the Pig Research Centre, Vietnam. After weaning at the age of 8 weeks, all the piglets were moved into the same animal house with concrete floor and natural ventilation. Nine pens were used for locating the animals. The piglets were separated into 9 groups according to breeds and fed *ad libitum* on standard commercial pig diets. The piglets were given one week to adapt to the new environmental conditions before they were immunised and bled.

3.2.2 Immunisation and other experimental procedures

Immunisation : All piglets, at 10 weeks of age, were immunised intramuscularly into the neck with 10 μg ovalbumin grade V (OVA; Sigma Chemical Co.) and 5μg keyhole limpet hemocyanin (KLH) Type VIII (Sigma Chemical Co.) together in a volume of 1ml phosphate buffered saline (PBS, 0.01 M, pH 7.4).

Blood sampling : Blood were drawn from the anterior vena cava into vacutainers containing either no additives or EDTA (Becton. Dickinson. USA). Serum was separated from clotted blood and stored at -20°C until use. EDTA-blood samples were used for haematology such as total leucocyte (white blood cell; WBC) count and leucocyte differential counts. For total WBC count, a Coulter electronic counter was employed. For differential WBC counts, two smears were prepared from each sample and stained with Diff Quick (Bacto

Laboratory, Sydney). One hundred cells were classified on each smear for differential WBC counts. From these data, the percentage of lymphocytes, neutrophils, eosinophils and monocytes were calculated for each animal.

Cell-mediated immunity (CMI) - skin reactivity : Piglets were sensitised to dinitrochlorobenzene (DNCB, Gibco BRL) by epicutaneous application of 100µl DNCB (5% DNCB in 95% ethanol mixed with 4:1 acetone and olive oil) to the skin of the inner left thigh. The skin was then challenged 14 days later by topical application of 100 µl DNCB (2.5%) in acetone: olive oil (4:1) and the double fold skin thickness was measured at 0, 24, 48 and 72 hours with a pair of microcalipers gauge (Mitutoyo MFG.Co.LTD.Tokyo.Japan) calibrated to 0.01 mm. The results were expressed as the difference between pre- and post -injection skinfold thickness. The piglets were also subjected to mitogenic challenge by intradermally injecting 20 µl of phytohaemaglutinin (PHA-M, Gibco BRL) in 100 µl saline to the skinfold. Two sites of skinfold on the belly of the animal were used (duplicate measurements). A "negative" control site which received only 100 µl of PBS was used. At 0, 24, 48 and 72 hours, skinfold thickness was measured as described above.

3.2.3 Enzyme-linked immunosorbent assays (ELISA) for antibodies against KLH and OVA

Two ELISAs were conducted in the Parasitology Laboratory, National Institute of Veterinary Research, Hanoi-Vietnam, for detecting primary and secondary

antibody responses to KLH and OVA on days 0, 7, 14, 21, 28, 35 and 42 of the immunisation schedule. Briefly, wells of flat bottom microtitre plates (Dade Diagnostics, Acacia Ridge, Pty.Ltd.,QU.4110) were coated with 100 µl antigen (either 5 µg/ml KLH or 5 µg/ml OVA) and incubated overnight at 4°C. The plates were washed 3 times with phosphate buffered saline containing 0.05% Tween-20 (PBST, pH 7.4). One hundred µl of standard sera or serum samples diluted with PBST to 1:200 for the primary immunisation and to 1:500 for the secondary immunisation were added. The plates were incubated for 1 hour at 37°C and washed 3 times with PBST. A rabbit anti-pig immunoglobulin conjugated with peroxidase (DAKO A/S, Denmark) was diluted 1:1000 in PBST and dispensed into each well. The plates were then incubated at 37°C for 1 hour, and washed 3 times with PBST. One hundred µl of OPD substrate (0.34% o-phenylenediamine in a citrate phosphate buffer, pH 6.0, containing 0.05% H_2O_2) was added to each well. The plates were incubated in dark at room temperature for 30 minutes before the reaction was stopped with 1 M H2SO4 (50 µl/well). The absorbance was measured at 492 nm with an automatic ELISA reader (Titertek Multiskan, Lab Systems, Finland). Absorbance was compared with values obtained from a standard curve and antibody titres were calculated using a curve- fitting programme based on an algorithm for least -squares estimation of non- linear parameters and transformed into values of $\log_2$ (antibody titre).

3.2.4 Schedule for experimental procedures

The schedule for immunisations and other experimental procedures is summarised as follows:

Day	Experimental Procedures
1	Blood collection for total WBC counts and differential WBC counts
2	PHA injection for skin test
3	Double skin thickness measured at 24 h
4	Double skin thickness measured at 48 h
5	Double skin thickness measured at 72 h
6	DNCB sensitisation
10	First immunisation with KLH and OVA. Blood samples for antibody response
17	Blood samples for antibody response
20	DNCB challenge (second immunisation)
21	Double skin thickness measured at 24h
22	Double skin thickness measured at 48h
23	Double skin thickness measured at 72h
24	Blood samples for antibody response
31	Blood samples for antibody response
38	Second immunisation with KLH and OVA
45	Blood samples for antibody response
52	Blood samples for antibody response

3.3.5 Statistical analysis

Data transformation was described in either Materials and Methods or Results. All data were analysed using two way analysis of variance (a generalised least square analysis program; REG:Gilmon, 1986) and least significant difference for group comparison. Due to the small number of dams involved, this variable was not included in the analysis but other effects were tested, including sex, breed and sire and their interactions. Only probability levels at $P<0.05$ were considered to be significant.

3.3 RESULTS

Antibody responses : Significant differences between breeds were found in the antibody responses to KLH both after primary and secondary immunisations. The Mong Cai breed produced significantly higher anti-KLH antibody response than the other two exotic breeds ($P<0.05$ to $P<0.001$), whereas no difference was seen between these two exotic breeds in their antibody levels (Table 3.1). No significant breed differences were found when animals were immunised with ovalbumin after the first or the second booster (Table 3.2). No significant effects were found in relation to sire and sex groups within each breed.

Table 3.1. Mean antibody response to KLH for the three pig breeds

Results are expressed as mean ± SEM of log_2 (antibody titre).

Time of measurement (days)	Mong Cai	Landrace	Yorkshire	
0 (first immunisation)	4.5 ± 0.28	3.9 ± 0.25	4.3 ± 0.31	ns
7	7.8 ± 0.56	5.9 ± 0.42	6.2 ± 0.51	ns
14	12.5 ± 1.18	8.7 ± 0.83	9.6 ± 0.78	***
21	10.7 ± 0.98	7.2 ± 0.89	6.8 ± 0.72	**
28 (second immunisation)	8.3 ± 0.85	5.8 ± 0.46	5.5 ± 0.39	*
35	14.8 ± 1.45	10.6 ± 1.02	12.3 ± 1.18	**
42	11.5 ± 1.05	7.8 ± 0.89	7.5 ± 0.87	*

ns: not significant *P<0.05 **P<0.01 ***P<0.001 significant differences between Mong Cai and the other two breeds.

Table 3.2. Mean antibody response to ovalbumin for the three pig breeds
Results are expressed as mean ± SEM of log_2 (antibody titre).

Time of measurement (days)	Mong Cai	Landrace	Yorkshire	
0 (first immunisation)	3.4 ± 0.28	4.1 ± 0.25	3.2 ± 0.30	ns
7	5.7 ± 0.49	5.9 ± 0.47	5.6 ± 0.45	ns
14	9.2 ± 0.98	8.9 ± 0.90	8.5 ± 0.96	ns
21	6.8 ± 0.65	6.5 ± 0.72	6.4 ± 0.58	ns
28 (second immunisation)	5.2 ± 0.55	5.6 ± 0.64	5.1 ± 0.5	ns
35	10.8 ± 1.12	10.2 ± 0.98	9.7 ± 0.88	ns
42	7.1 ± 0.75	7.8 ± 0.91	7.2 ± 0.71	ns

ns : not significant

Haematology : The total and differential WBC counts were found to be within the normal range, although a significant breed variation was identified. As shown in the Table 3.3, significant breed differences were observed in total WBC counts and the percentages of eosinophils and monocytes ($P<0.05$). The total WBC and eosinophil counts were found to be significantly higher ($P<0.05$) in the Mong Cai breed (23.03 ± 0.8) than those in Yorkshire (20.06 ± 0.6) and Landrace (19.43 ± 0.9), whereas the monocyte count in the Mong Cai breed was relatively lower than those in the two exotic breeds ($P<0.05$). No significant effects were found in relation to sire and sex groups within each breed.

Table 3.3 Total and differential WBC counts of three pig breeds. Results are expressed as mean ± SEM of counts

Parameters	Mong Cai	Landrace	Yorkshire	
WBC (10^6/ml)	23.0 ± 0.8	19.4 ± 0.9	20.1 ± 0.6	*
Lymphocytes (%)	59.4 ± 0.7	61.3 ± 0.5	63.9 ± 0.7	n.s
Neutrophils (%)	34.5 ± 0.6	32.2 ± 0.9	31.7 ± 0.5	n.s
Eosinophils (%)	2.9 ± 0.2	2.1 ± 0.3	1.8 ± 0.2	*
Monocytes (%)	3.4 ± 0.1	4.2 ± 0.2	4.5 ± 0.3	*

n.s: not significant * P<0.05 significant difference between Mong Cai and the other two breeds

Skin tests : No significant changes in skinfold thickness were noted at saline injection sites. In contrast, intradermal injections of PHA induced a strong local skin reaction as indicated by an increase in skinfold thickness. The reaction reached a maximum at 24 hours after immunisation and gradually declined at 48 and 72 h (Figure 3.1). The skin reaction in response to DNCB challenge also had a similar pattern as PHA-induced reaction, where the peak reaction was at 24 hr after challenge. Using the increase in skinfold thickness for analysis, there was a significant difference ($P<0.05$) between the Mong Cai breed and the two exotic breeds at 24 hr (their maximum responses) (Table 3.4). The DNCB reaction was however significantly different ($P<0.05$ to $P<0.01$) between the Mong Cai breed and the two exotic breeds (Table 3.5). No significant effects were found in relation to sire and sex groups within each breed.

(Results of all the above experiments which indicated no significant sire variation within individual breeds are shown in the Appendix.)

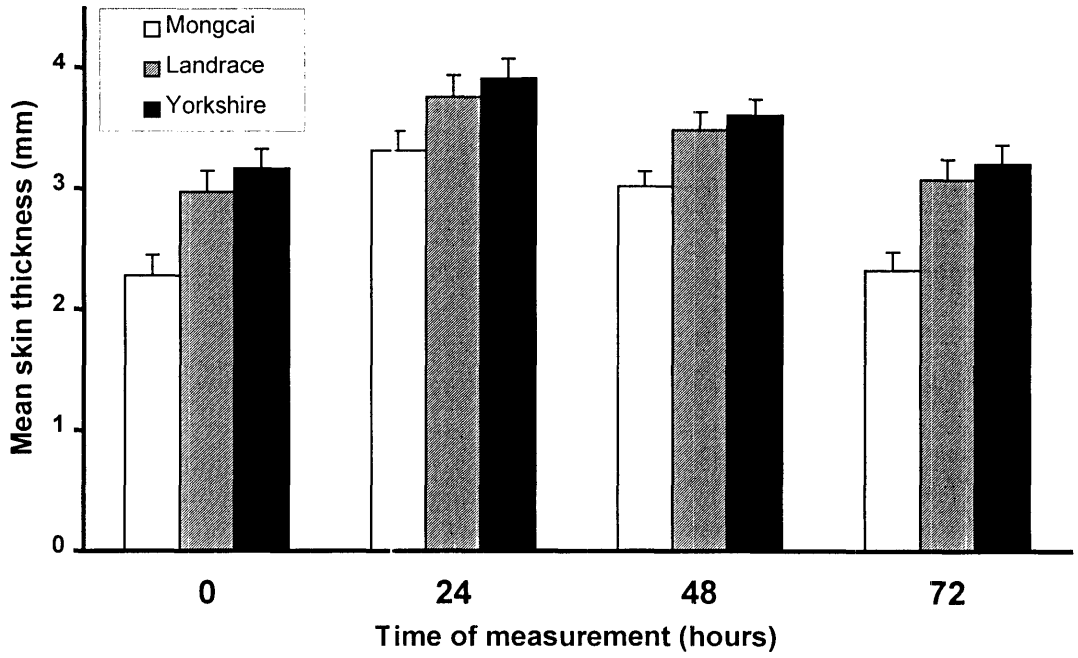

Figure 3.1 Double fold skin thickness reaction to PHA of three pig breeds. Results are expressed as mean ± SEM of thickness (mm)

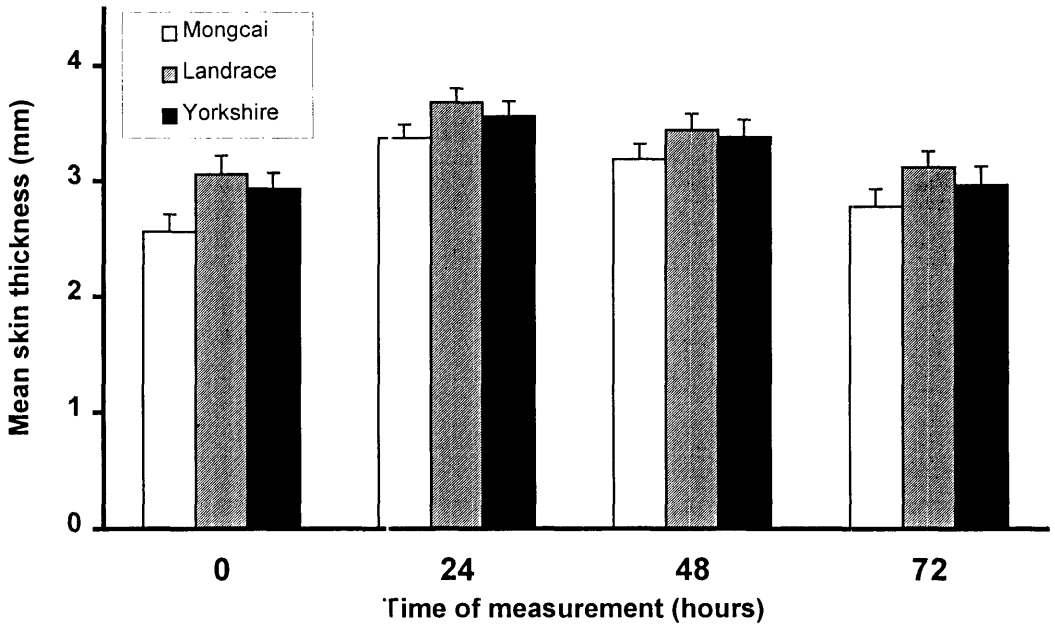

Figure 3.2 Double fold skin thickness reaction to DNCB of the three pig breeds. Results are expressed as mean ± SEM of thickness (mm)

Table 3.4 Mean increase in skin thickness in response to PHA of the three pig breeds. (Results are expressed as mean ± SEM of thickness-mm)

Time of measurement (hours)	Mong Cai	Landrace	Yorkshire	
24	0.81 ± 0.17	0.62 ± 0.18	0.59 ± 0.08	*
48	0.63 ± 0.19	0.53 ± 0.17	0.45 ± 0.13	ns
72	0.29 ± 0.06	0.24 ± 0.07	0.31 ± 0.11	ns

ns: not significant *P<0.05

Table 3.5 Mean increase in skin thickness in response to DNCB of the three pig breeds. (Results are expressed as mean ± SEM of thickness-mm)

Time of measurement (hours)	Mong Cai	Landrace	Yorkshire	
24	1.04 ± 0.23	0.86 ± 0.19	0.74 ± 0.21	**
48	0.74 ± 0. 24	0.62 ± 0.13	0.54 ± 0.15	*
72	0.25 ± 0.03	0.38 ± 0.07	0.29 ± 0.06	*

* P<0.05 ** P<0.01

3.4 DISCUSSION

Recently, Edorfs-Lilja et al (1994) have clearly demonstrated that the heritability estimates for total WBC counts, mitogen (Con A) -induced proliferative response of lymphocytes, production of cytokines such as interferon-γ and interleukin-2 are sufficiently high to be used as genetic markers in selection for superior immune competence in pigs. In addition to the commonly used humoral antibody response, this study has selectively employed the above cell-mediated immune responses to assess the genetic variation in three different breeds raised in Vietnam. As piglets may suffer from temporary immunosuppression and substantial maternal influence during weaning period while the immune system has not become fully mature, the piglets used in this study were all above 8 weeks old, when most immune parameters supposedly reach the adult status (Gonzalez et al, 1993).

Results from this study demonstrated a general superiority of the indigenous Mong Cai breeds over the other two exotic breeds (Yorkshire and Landrace) in most humoral and cellular immune parameters which might account for their general immune competence. However, there appeared to be no genetic variation in immune status within individual breeds. It is interesting to note that while a significant breed difference in the ability to produce anti-KLH antibody was observed, there seemed to be no difference between breeds in anti-ovalbumin antibody levels. This might result from a genetic variation at

the antigenic recognition level for these two different immunogens. This is not unusual as antigenic recognition is well under genetic control (Lie, 1990). If so, it is possible to speculate their variation in responding to pathogenic challenge. Similar studies were also reported by Joling et al (1993), who indicated a significant variation between Yorkshire and Landrace breeds in the antibody response to KLH. The discrepancies between Joling et al's findings and the present findings reflect that genetic variation within breeds may exist in these two exotic breeds although non-genetic influencing factors including the methodology of assessment are not excluded. Antibody responses to novel antigens such as erythrocytes and ovalbumin have already been shown to have association with genetic improvement of disease resistance (Burton et al., 1989). Whether the superiority of Mong Cai in their antibody responses would suggest their superiority in disease resistance remains to be investigated.

Results from this study showed significantly higher total white blood cell, monocyte and eosinophil counts in the indigenous breed, Mong Cai. The total white blood cell count has been frequently used by other researchers to study genetic variation between breeds in immune competence and its association with disease resistance in pigs (McTaggart and Rowntree,1969; Buschman et al., 1982). Similar to the present findings, variation in blood monocyte count and eosinophil count between different breeds of pigs was also reported by Buschman et al. (1982). As suggested by Buschman et al. (1982), the difference in the number of leucocytes might reflect the level of disease resistance in animals that are bred in the same environmental conditions.

Therefore, the present haematological results again give support to the superiority of Mong Cai breed over the other exotic breeds in terms of disease resistance. However, assessing the genetic variation in the functional aspects of white blood cells would be of great importance.

The cell-mediated immune response was demonstrated in this study using both *in vivo* mitogen- (PHA-) induced cellular response and contact (delayed-type) hypersensitivity response. Both study models had a common target of assessing T lymphocyte functional response. While PHA non-specifically or polyclonally stimulated T cell proliferation, the DNCB-elicited response was a specific T cell-mediated hypersensitivity reaction (Titus and Chiller, 1981). The Mong Cai breed again demonstrated a stronger reaction than the other two breeds. The findings are suggestive of the superiority of T cell responsiveness of Mong Cai. As T cells play a significant role in initiating and regulating the cell-mediated immunity against many infectious and non-infectious diseases, the superiority of Mong Cai in T cell responsiveness may offer this breed a better immune competence in combating diseases. Although significant variation in lymphocyte functions including cytokine production have been found between the Yorkshire breed and others such as Dutch and Norwegian Landrace breeds as well as between different lines of the Yorkshire breed (Joling et al, 1993; Mallard, et al., 1982; Reddy et al, 1995), no significant superiority of this breed was obeserved as compared to Mong Cai and Landrace. This again suggests that the possible presence of genetic variation in the immune status of different populations of the Yorkshire breed.

In conclusion, there is genetic variation in immune competence between the populations of the three breeds evaluated in this study The indigenous breed Mong Cai,population consistently showed superiority over the other two exotic breeds, Yorkshire and Landrace, in both humoral and cell-mediated immune responsiveness. By comparing studies by other researchers, without disregarding the possible involvement of non-genetic factors, it is possible to speculate that genetic variations in immune competence within the two exotic breeds may also be present. To address this question, in the next chapter, the genetic variation in immune competence of two popular Australian pig breeds (Large White and Duroc) is discussed. Genetically, Large White and Yorkshire are in fact identical at the breed level. Overall, this study has warranted further investigation into the superiority of disease resistance in Mong Cai pigs.

Chapter 4

VARIATION IN IMMUNE COMPETENCE OF TWO PIG BREEDS IN AUSTRALIA

4.1 INTRODUCTION

Chapter 3 has clearly demonstrated some possible genetically-linked variations in immunological parameters. Apart from genetic influence, there are a number of non-genetic factors which also have direct or indirect effects on the immune system of animals and may be associated with the genetic components (Siegel and Gross, 1978). Influences arising from environmental, behavioural, managerial differences probably amount to some major non-genetic factors which are constantly interacting with genetic components and affect the immune competence of pigs (Hessing et al, 1994 and 1995). In view of this, although there are many reports on genetic variations in disease resistance in the pig breeds that Australia also commonly use for our industry, it is often important to study the unique genetic and non-genetic features of the immune status of certain populations of these breeds that have been bred and raised within Australia for many generations.

In livestock breeding, monitoring immune status has become increasingly important as selection of superior production traits should not incur any

adverse effects on animals' immune competence, immunity against diseases or immune responsiveness to vaccination. As reviewed in Chapter 2, studying immune competence has provided an alternative to evaluate genetic resistance to disease without damaging important production traits or engaging economically costly disease challenge trials. The establishment of a suitable and relevant immune competence profile should embrace measures of both humoral and cell-mediated immune responses which orchestrate the major protective mechanisms against infectious diseases. In this regard, an immune competence profile for pigs has been developed by Buschman and Meyer (1989), who reported that most parameters tested had a positive correlation with disease resistance. Good markers for immune competence was believed to include humoral responses to novel antigens or vaccine challenge, a numbers of various leucocyte populations and distribution of various T-cell sub-populations (Buschman and Meyer, 1989). Of interest, genetic differences in the humoral and cellular immune responses of pigs to different antigens or vaccines using sheep erythrocytes, *Bordetella brochiseptica* and pseudorabies and *E.coli* have been previously reported (Buschman et al., 1974; Rothschild, et al., 1984a & b; Edfors-Lilja, 1991). Significant variations among four different pig breeds were also detectable in both *in vitro* and *in vivo* lymphocyte proliferative response and antibody response to novel antigens such as KLH (Joling et al., 1993). In Chapter 3, we have demonstrated the superiority of an indigenous breed of pigs over the other exotic breeds raised in Vietnam. The present study was however designed to investigate the possible variation in the immune competence of two pig breeds, Large White and

Duroc, which have been widely raised in Australia for years. In particular, the focus of this study has been placed on genetic variation in lymphocyte functional characters and immune responsiveness to vaccine challenge.

4.2 MATERIALS AND METHODS

4.2.1 Animals

Pigs were kindly supplied by Pig Research Centre, Animal Science Institute, the Queensland Department of Primary Industries, Brisbane, Queensland. A total of 48 piglets of both sexes arising from 12 sows and 9 boars of either Large White or Duroc were used. Two Duroc boars and one Large White boar sired two litters and the other six boars sired only one litter each (Table 1) . Piglets were born between 4 January and 8 January, 1996 at the Pig Research Centre. After weaning at 4 weeks old, all piglets were moved to the same animal house. Piglets were allocated to each pen with equal numbers of both sexes. Animals were fed *ad libitum* on standard pig diets for weaners. All piglets were subjected to the same environmental conditions throughout the entire study.

Table 1. Numbers of piglets per sire

Breed	**Sire Group**	**Number**
Duroc	1	4
Duroc	2	8
Duroc	3	4
Duroc	4	8
Large White	5	8
Large White	6	4
Large White	7	4
Large White	8	4
Large White	9	4

4.2.2 Blood sampling

The blood samples were collec ed taken four times over a period of 3 months before and after vaccination. The first blood sample was taken one week before vaccination at the age of 5 weeks. Fourteen days after the first vaccination, a second blood sample was taken (7 weeks old). The third and the fourth blood collections were taken at 7 and 14 days after the second vaccination (11 and 12 weeks old respectively). With the final sample, an average of body weight of 40 kg was observed. All the pigs appeared to be clinically normal at all times throughout the entire study.

4.2.3 Vaccination

One week after weaning (5 week old) all the piglets were vaccinated with "Lepto for Pigs" vaccines (CSL, Victoria, Australia) against *Leptospira interrogans* servars *pomona and tarassovi*. A dose of 2 ml of each vaccine was subcutaneously injected into the piglets. A booster was applied 4 weeks later using the same dosage.

4.2.4 Haematological analysis

Blood was taken from the jugular vein into vacutainer tubes containing either EDTA or heparin (Becton Dickinson. USA). With EDTA-blood, the total leucocyte or white blood cell (WBC) counts, red cell counts and pack cell volume (PCV) were measured using a Coulter electronic counter. Differential WBC counts were done using blood smears as described previously.

4.2.5 Lymphocyte stimulation test (LST)

Whole blood culture using heparinized blood was applied in LST. A whole blood culture for LST was set up to optimise the dilution factor for whole blood and dose for T cell mitogen, Concanavalin A (Con A). Whole blood was found to be optimal after 1:40 dilution while Con A was 5 μg/ml (final concentration per well - these preliminary data are not shown). RPMI 1640 (Sigma Chemical Co. USA) containing penicillin G (100 units/ml), streptomycin (100 μg/ml) was used as culture medium for LST. A volume of 200 μl of diluted whole blood was placed into each well of sterile flat-bottomed 96-well plates and 20 μl of Con A at 50 μg/ml (this gave a fnal dose of 5 μg/ml). All cultures were set up in triplicate. After incubation for 72 hours at 37°C in a humidified atmosphere containing 5% CO_2-in-air, 20 μl of RPMI-1640 containing 12.5 μCi/ml of [3H]-thymidine (Amersham, England, UK) were added to each well. Eighteen hours later, using a Titertek Plus Cell Harvester (ICN, USA), cells were harvested onto filter papers (ICN). The

filters were air-dried and the incorporation of [3H]-thymidine for each culture was determined in a liquid scintillation counter (LKB, Finland). The mean of counts per minute (cpm) for each set of culture was calculated and the results were expressed as stimulation indices (SI) (= cpm of unstimulated culture / cpm of Con A-stimulated culture).

4.2.6 Fluorescence activated cell sorter (FACS) analysis for lymphocyte phenotypes

A standard procedure for FACS staining which was established at CSIRO Immunology Laboratory, Pastoral Research Laboratory, Chiswick, Australia, was used in this study. Monoclonal antibodies against $CD2^+$, $CD4^+$ and $CD8^+$ lymphocyte subsets were generously provided by the Centre for Animal Biotechnology, School of Veterinary Science, University of Melbourne, Australia. To distinguish $CD4^+/CD8^+$ cells from others, a double-staining procedure using both fluorescein isothiocyanate (mAb-FITC; 1/200) and anti-mouse Ig conjugated with phycoerythrin (mAb-PE; 1/250) (Silenus, Australia) was employed. EDTA-blood samples were used for FACS analysis. The total WBC counts were performed as described for haematological studies. Seven tubes were labelled for each blood sample. They included three control tubes for blood cells only, mAb-FITC and mAb-PE respectively and four test tubes for $CD2^+$, $CD4^+$, $CD8^+$ and $CD4^+CD8^+$ respectively. Based on the counts of leucocyte, the amount of whole blood to be added was : 100 µl for count of 0 - 6 x 10^9; 75µl for 6 - 10 x 10^9 ; 50µl for 10 - 20 x 10^9 and 25µl for more than

20×10^9. The amount of monclonal antibodies added depended on the volume of blood in the tube : 25 µl for 50 µl whole blood, 37 µl for 75 µl whole blood and 50 µl for 100 µl whole blood. After adding appropriate volumes of whole blood and CD marker-specific monoclonal antibodies, the tubes were flicked and incubated for 20 minutes on ice in the dark. After this 2 ml of phosphate buffered saline containing 0.05% sodium azide (PBS/azide) was added to each tube, and the tubes were centrifuged at 1400 rpm at 4°C for 5 minutes. The supernatant was discarded. Samples were washed again with PBS/azide before mAb-FITC and/or mAb-PE were added to each tube. The amount added was equal to the amount of the first CD marker detecting mAbs. The tubes were flicked and incubated for another 20 minutes on ice in the dark. After this step, to lyse the unwanted red blood cells, 100 µl of formalin (7 - 8 %) in PBS was added to each tube, flicked and allowed to act for 1 -2 minutes. One ml of milli-Q water was then added to each tube which were then flicked and placed in a 37°C waterbath for 3 - 5 minutes. The red cells were checked for complete lysis, and if this had not occurred the tube was returned to the waterbath for up to 10 minutes. After the lysis was confirmed, 2 ml of PBS/azide was added to the tubes and the samples mixed and then centrifuged at 1400 rpm at 4°C for 5 minutes. The supernatant was discarded and the step repeated once before 200µl of FACS-fix (containing 1% paraformaldehyde) was added to each tube. The cells were then ready for analysis; however, they were routinely stored at 4°C until analyses were performed on the following day using a flow cytometer (FACS Vantage, Becton Dickinson, USA).

The data were then analysed and expressed as percentages of each CD^+ sub-population. The absolute number of each of these CD positive cells was converted based on their absolute lymphocyte counts.

4.2.7 Direct ELISA for serum IgG level.

Sera from jugular venous blood were separated and stored at -20°C for IgG level analysis. A direct ELISA for IgG was employed to analyse IgG level in serum samples. Briefly, standard ELISA microtitre plates (Immulon II, Dynatech, USA) were coated with 100 µl of purified pig IgG (Sigma USA) at various concentrations (40; 10; 2.5; 0.625; 0.156; 0.039; 0.01 or 0.0025 µg/ml which served as a standard curve). Each test serum was 4x-serially diluted with an initial dilution of 400x, and each dilution was set up in triplicate. Carbonate-bicarbonate buffer (pH 9.6) was used as coating buffer and serum diluting buffer. Plates were coated overnight at 4°C and then washed with PBST (0.05% Tween-20 in PBS, pH 7.4) four times before addition of 100 µl of rabbit anti-pig IgG-peroxidase conjugate (1:30,000; Sigma USA) in PBST. The plates were then incubated at 37°C for one hour and washed four times with PBST before subjected to 100 µl/well of freshly prepared substrate (0.34% o-phenylenediamine in a citrate phosphate buffer, pH 6.0, containing 0.05% H_2O_2). The reaction was allowed to develop for 20 minutes in the dark at 37°C and then stopped by 50 µl / well of 1M H_2SO_4. Absorbance (opitcal density; OD) was measured using a Titertek Multiskan reader (Flow Laboratories, USA) at a wavelength of 492 nm. The OD results were

subjected to log-transformation, and serial concentrations of standard porcine IgG were used to produce a standard curve using linear regression for evaluating the final concentration of each test sample.

4.2.8 Microscopic agglutination test (MAT) for serum antibodies against *Leptospira interrogans*

The MAT was conducted by the WHO-FAO Leptospirosis Reference Laboratory, State Health Laboratories, Brisbane, QLD. Briefly, all sera were spun in a microcentrifuge to remove contaminating particles prior to testing. Serum samples were diluted 1/25 in PBS (pH 7.2) and mixed well for testing. Twenty five µl of diluted test serum was added to each well (in quadriplicates) of a flat bottomed microtitre plates. Testing was performed for each serovar type (*pomona and tarassovi*) using both positive and negative sera as control, autoagglutination of the antigen was also controlled by using saline alone. After addition of another 25 µl of PBS and 25µl of each antigen to all wells, the plate was covered and incubated at 37°C for 60 minutes. The plate was removed from incubator and each well was examined for agglutination at a magnification of 40x with an aid of a dark field illuminator. All plates were read within one hour after incubation. Results are expressed as ranks of titre.

4.2.9 Statistical analysis

Data transformation was described in either Materials and Methods or Results. All data were analysed using either a generalised least square analysis program or two-way analysis of variance with Student-Newman-Keuls test (parametric or non-parametric) for multiple comparisons. Mann-Whitney Rank Sum test was for comparing data obtained from MAT. Comparisons were made as breeds versus gender and sires versus genders. Due to the small number of dams involved, this variable was not included in the analysis but other effects were tested, including sex, breed and sire and their interactions. Probability levels at $P<0.05$ were considered to be significant.

4.3 RESULTS

Haematological results: Table 4.1 presents the results of total and differential leucocyte counts. There was no significant difference in total WBC counts between the two breeds before the vaccination. After the first and second immunisation, the number of total counts had increased within either breed ($P<0.01$). However, Large White piglets consistently showed significantly higher WBC counts than the Duroc piglets at the two time points ($P<0.05$) following either the first or the second immunisation.

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. Red blood cell counts, monocyte counts and packed cell volume (PCV) were within the normal range for pigs and remained unchanged before and post immunisations between the two breeds (Table 4.1) although significant increases in red cell counts and PCV were observed within each breed after the immunisations .

Neither sire (within breed) nor sex (within and between breeds) had significant effects on the above haematological data.

Table 4.1 Haematological analysis of the two pig breeds

Results are expressed as (mean ± SE)

Item - time - unit	Duroc	Large White	
WBC 1 ($x10^6$ / ml)	10.0 ± 0.3	10.6 ± 0.2	ns
WBC 2 ($x10^6$ / ml)	16.8 ± 0.5	20.5 ± 0.4	*
WBC 3 ($x10^6$ / ml)	18.6 ± 0.4	22.4 ± 0.6	*
Lymphocytes 1 (%)	55.5 ± 0.8	54.1 ± 0.5	ns
Lymphocytes 2 (%)	47.7 ± 0.6	50.1 ± 0.7	ns
Lymphocytes 3 (%)	41.3 ± 0.7	45.4 ± 0.8	ns
Neutrophils 1 (%)	40.6 ± 0.9	41.8 ± 0.6	ns
Neutrophils 2 (%)	50.4 ± 0.7	46.8 ± 0.8	*
Neutrophils 3 (%)	54.8 ± 0.5	49.6 ± 0.9	**
Eosinophils 1 (%)	1.5 ± 0.2	1.6 ± 0.1	ns
Eosinophils 2 (%)	0.5 ± 0.1	1.3 ± 0.3	*
Eosinophils 3 (%)	1.8 ± 0.3	1.9 ± 0.2	ns
Monocytes 1 (%)	2.2 ± 0.2	2.7 ± 0.3	ns
Monocytes 2 (%)	1.2 ± 0.1	1.7 ± 0.2	ns
Monocytes 3 (%)	1.5 ± 0.3	2.8 ± 0.4	ns
Red cell counts 1 ($x10^6$/ml)	6.9 ± 0.08	6.8 ± 0.09	ns
Red cell counts 2 ($x10^6$/ml)	6.7 ± 0.08	7.3 ± 0.06	ns
Red cell counts 3 ($x10^6$/ml)	7.1 ± 0.05	7.1 ± 0.07	ns
PCV1 (%)	30.2 ± 0.7	32.4 ± 0.5	ns
PCV 2 (%)	34.4 ± 0.6	34.9 ± 0.8	ns
PCV 3 (%)	39.6 ± 0.7	40.5 ± 0.9	ns

ns : not significant (between breed); * $P< 0.05$ (between breeds)

** $P<0.01$ (between breeds)

1 = 7 days before immunisation; 2 = 14 days after first immunisation;

3 = 7 days after second immunisation

Lymphocyte stimulation test (LST) results: As daily variation in LST within individuals is usually high, the stimulation index (SI) which has proven useful was employed in this study for analysis. The results revealed that there was only a significant difference ($P<0.05$) between the two breeds before immunisation - Large White pigs showed higher lymphocyte responsiveness to Con A stimulation than Duroc pigs (Table 4.2). Neither sire (within breed) nor sex (within and between breeds) had significant effects on lymphocyte stimulation induced by Con A.

Table 4.2 Con A-induced lymphocyte stimulation of the two pig breeds

Results are expressed as mean ± SEM of stimulation index.

	Duroc	**Large White**	
7 days before vaccination	8.3 ± 3.4	12.6 ± 4.2	*
14 days after I vaccination	23.8 ± 7.2	31.7 ± 9.3	ns
7 days after II vaccination	93.4 ± 25.3	100.4 ± 26.1	ns

ns : not significant * $P<0.05$

FACS analysis of lymphocyte phenotypes : No significant difference was found in the relative distribution (%) of each phenotype within and between breeds at all times during the study as shown in Table 4.3. However, when the absolute number of each phenotype was used for analysis, significant increases ($P<0.05$) in all phenotypes within each of the two breeds were noticed after each of the two immunisations. Between breeds, the $CD2^+$ and $CD4^+$ cells were found significantly different at both time points after the first and second immunisations. Large White showed significantly higher numbers of $CD2^+$ and $CD4^+$ cells ($P<0.01$ and $P<0.05$ respectively) than Duroc (Table 4.4). Of interest, there were significant sire effects ($P<0.05$) on CD8+ cells within the Large White breed at 7 days after the first immunisation (Table 4.5). Sire group 7 and 9 had higher $CD8^+$ cell levels than sire group 5 and/or 8. No sex effect on any phenotypes of lymphocytes was observed between or within breeds throughout the study.

Table 4.3 The proportion (mean % ± SEM) of the various lymphocyte subsets of the two pig breeds

	CD2$^+$	CD4$^+$	CD8$^+$	CD4$^+$/CD8$^+$
Duroc				
7 days before 1st vaccination	53.9 ± 1.5	22.9 ± 1.3	21.7 ± 1.2	0.8 ± 0.1
14 days after 1st vaccination	49.8 ± 2.2	18.8 ± 0.8	21.9 ± 1.8	2.2 ± 0.2
7 day after 2nd vaccination	52.7 ± 1.9	17.5 ± 0.9	23.8 ± 1.6	3.6 ± 0.5
Large White				
7 days before 1st vaccination	49.3 ± 2.7	21.3 ±0.9	18.7 ± 1.3	0.7 ± 0.1
14 days after 1st vaccination	53.3 ± 1.7	23.9 ±0.7	20.8 ± 0.9	2.6 ± 0.3
7 day after 2nd vaccination	57.1 ± 1.4	21.8 ±1.2	21.3 ± 2.1	2.5 ± 0.4

Table 4.4 The absolute counts ($x10^6$/ml; mean ± SEM) of the various lymphocyte subsets of the two pig breeds

	CD2$^+$	CD4$^+$	CD8$^+$	CD4$^+$/CD8$^+$
Duroc				
7 days before 1st vaccination	2.9 ± 0.3	1.3 ± 0.1	1.2 ± 0.2	0.07 ± 0.01
14 days after 1st vaccination	4.0 ± 0.3*	1.4 ± 0.1**	1.8 ± 0.2	0.18 ± 0.02
7 day after 2nd vaccination	5.3 ± 0.4*	1.7 ± 0.1**	2.6 ± 0.3	0.20 ± 0.03
Large White				
7 days before 1st vaccination	2.9 ± 0.3	1.2 ± 0.1	1.2 ± 0.2	0.08 ± 0.01
14 days after 1st vaccination	5.4 ± 0.3*	2.4 ± 0.1**	2.5 ± 0.3	0.18 ± 0.02
7 day after 2nd vaccination	6.7 ± 0.4*	2.3 ± 0.1**	3.2 ± 0.3	0.30 ± 0.03

*P<0.05 ,**P<0.01 (significance between breeds of the same lymphocyte subsets.)

Table 4.5 The number of $CD8^+$ cells for each sire within breed at 7 days after the initial vaccination.

Breed	Sire group	Mean	SEM
Duroc	1	710.26	166.29
Duroc	2	893.04	117.59
Duroc	3	1043.11	166.29
Duroc	4	473.00	117.59
Large White	5	651.48	73.70
Large White	6	832.85	104.23
Large White	7* #	1176.47	104.23
Large White	8	739.85	104.23
Large White	9*	1123.47	104.23

Significant differences ($P<0.05$) between sire groups were found within the Large White breed. * significant different from group 5; # significant different from group 8.

Serum IgG levels : The results are shown in Table 4.6. Although there was a significant increase ($P<0.05$) after the immunisations within each breed. No differences between breeds or sexes were detectable in the IgG levels, but there were sire differences within each breed before the initial vaccination ($P<0.05$) (Table 4.7). Sire groups 1 and 8 had significantly higher serum IgG levels than all other sire groups within the same breed whereas sire group 2 was also found higher than group 4 in serum IgG.

Table 4.6 Serum IgG levels (μg/ml) of the two pig breeds. Results are expressed as mean ± SEM.

	Duroc	**Large White**	
7 days before vaccination	823.6 ± 94.3	1098.1 ± 183.4	ns
4 days after I vaccination	1168.5 ± 101.6	1202.4 ± 165.4	ns
7 days after II vaccination	2212.6 ± 215.6	2632.1 ± 233.4	ns

ns : not significant.

Table 4.7 Serum IgG levels (μg/ml) of various sire groups within the two breeds 7 days before the initial immunisation.

(Results are expressed as mean ± SEM.)

Breed	Sire groups	Mean	SEM
Duroc	1*	1160.8	338.1
Duroc	2**	944.9	165.0
Duroc	3	687.2	176.3
Duroc	4	602.2	54.8
Large White	5	1037.4	284.6
Large White	6	664.1	161.1
Large White	7	744.3	168.2
Large White	8*	2560.2	428.6
Large White	9	545.2	62.3

Significant differences (P<0.05) between sire groups within each breed.

*** different from all the other groups within the same breed**

**** different from sire group 4**

Table 4.8 Serum antibody titres against two serova of *Leptospira interrogans* of the two pig breeds at 7 days after vaccination.

	Duroc *pomona*	**Duroc** *tarassovi*	**Large White** *pomona*	**Large White** *tarassovi*
7 days before vaccination	0	0	0	0
4 days after 1st vaccination	0	0	0	0
7 days after 2nd vaccination	0	1:50	1:50	0

Results are expressed as median of titres

Table 4.9 Serum antibody titres against two serova of *Leptospira interrogans* of different sire and sex groups within the two pig breeds at 14 days after the vaccination.

	Duroc *pomona*	**Duroc** *tarassovi*	**Large White** *pomona*	**Large White** *tarassovi*
Female	25	50	75	25
Male	0	25	25	0
Sire 1	75	0	N/A	N/A
Sire 2	0	0	N/A	N/A
Sire 3	75	100	N/A	N/A
Sire 4	25	25	N/A	N/A
Sire 5	N/A	N/A	100	0
Sire 6	N/A	N/A	0	0
Sire 7	N/A	N/A	150	100
Sire 8	N/A	N/A	25	25
Sire 9	N/A	N/A	00	0

Results are expressed as median of titres. N/A = Not Applicable

Antibody against Leptospira interrogans : The results are expressed as ranks of titre and are shown in Tables 4.8 and 4.9. No significant titre difference was found 7 days after vaccination but some titres were found to increase within each breed on 14 days. However, no significant variation was found in breeds, sires and sexes.

(Results of all the above experiments which indicated no significant sire variation within individual breeds are shown in the Appendix.).

4.4 DISCUSSION

From a genetic viewpoint, Large White pigs and Yorkshire pigs share a common ancestor whereas the former is in fact an improved breed from the latter in terms of some production traits. In some previous studies (Joling et al, 1993; Mallard, et al., 1991; Edfors-Lilja et al, 1994; Reddy et al, 1995), Yorkshire pigs showed significant genetic variation in their immunological responsiveness among different breeding lines and might have some superior immunological traits to other breeds. Although Mong Cai pigs demonstrated superiority over Yorkshire and Landrace in many immunological parameters as described in the previous chapter, Large White pigs (a very close relative of Yorkshire pigs) raised in Australia for generations showed significant superiority over Duroc in their immune responsiveness, and more interestingly,

it was obvious that certain sire lines might be responsible for the superiority in some cases.

There appeared to have general increases within each of two breeds in the immunological parameters such as white blood cell counts, lymphocyte phenotype counts, Con A-induced lymphocyte response and serum IgG. These were likely due to the change of age in animals as reported by (Jain, 1986; McTaggart and Rowntree, 1969), but the response of pigs to immunisations was also believed to be responsible.

The Yorkshire breed has previously shown a higher serum neutralising antibody titre level than the Duroc breed in response to immunisation with a pseudorabies modified live-virus vaccine (Rothschild et al, 1984c). This study however did not show any differences between the Large White and Duroc breeds in antibody responses to vaccination against *Leptospira interrogans*. However, the levels of serum antibody against *Leptospira interrogans* remained very low in animals after two immunisations. This could be due to the use of micro-agglutination test (MAT) which is generally considered to have better sensitivity if used to detect antibodies induced by natural infection rather than vaccination. T cells, especially T helper cells, play an important role in initiating both T cell-dependent antibody response and cell-mediated immune responses (Tizard, 1995). It is worth noting that although there was no significant difference between the two breeds in the serum antibody against

Leptospira interrogans, the significant difference in $CD2^+$ (a pan T cell surface marker) and $CD4^+$ (a T helper cell surface marker) cell counts between the two breeds occurring only after the immunisations might indirectly show a better immune responsiveness to vaccination in Large White pigs. Furthermore, many studies (Gill et al.,1993and 1994; Jankovic, et al, 1996) have reported that mechanisms underlying genetic resistance to gastrointestinal nematodes in sheep was significantly mediated by T helper (CD4) cells, and differences in susceptibility between genotypes might be due to differences in their T helper cell function. Whether the higher $CD4^+$ cell response of Large White pigs would render them better resistance mechanisms against gastrointestinal nematode resistance or other cell-mediated immune protection requires further investigation. In this regard, studying the function of T helper sub-population, particularly the possible Th1 and Th2 responses, and their cytokine profile should yield a better understanding of their potential in disease resistance.

In chicken, using breeding lines of high and low immune responders, Kreukniet et al., (1994) were able to demonstrate that the distribution of $CD4^+$ cells was higher in the high responder line, while the percentage $CD8^+$ cells was higher in the low responder line. Similarly, in this study, significantly higher $CD8^+$ cell levels of two sire lines than those of other lines in Large White pigs reflected the presence of genetic variation within breed in the immune responsiveness. As $CD8^+$ cells represent cytotoxic lymphocytes, whether the higher number of these cells would offer these particular sire lines

better efficiency in combating viral infections remains to be solved. The within-breed variation in serum IgG level was also noticed among different sire lines of each of the two breeds before any immunisations. However, in addition to sire effects, influence originating from dams might be possible in this study as dam effects on total immunoglobulin level of Yorkshire piglets from the same litters have recently been reported (Edfors-Lilja et al, 1994). Unfortunately, due to the small number of dams involved, the analysis of this factor was not included in this study. Pigs are unique with the presence of mature lymphocytes expressing $CD4^+/CD8^+$ in peripheral blood and other second lymphoid tissues (Pescovitz, MD, 1994). The immunophysiological function of these cells is still an enigma, but nevertheless, no significant difference was found either between or within the two breeds.

The use of whole blood culture for studying pig lymphocyte mitogenic responses in this study was reported for the first time and has proven useful. Although Large White pigs showed a pronounced difference in non-specific Con A-induced T lymphocyte proliferative responses before any immunisations, such difference was no longer observed after immunisations. This might indicate the likelihood of genetic variation between the two breeds in the impact of aging on cell-mediated immune responses although whether the immunisations had buffering effects on the change requires investigation.

Good markers for immune competence was believed to include humoral responses to novel antigens or vaccine challenge, a numbers of various leucocyte populations and distribution of various T-cell sub-populations (Buschman and Meyer, 1989). In this study, these responses had also been applied to compare two popular Australian breeds - Large White and Duroc. Large White pigs clearly demonstrated an overall superiority over Duroc.

Chapter 5

GENERAL CONCLUSIONS

A genetic basis to the differences that exist between and within breeds of a species with respect to their disease resistance and immunocompetence is well known. As reviewed in Chapter 2, genes which control the host immune response to a pathogen is closely related to the innate immunity; the specificity of adaptive immune responses and the quality of adaptive immune responses (Owen and Axford, 1991; Gavera and Spencer, 1983; Buschman et al., 1982; Warner et al., 1987). However, the expression of disease resistance in animals is not usually restricted to a single gene but rather it is also governed by accumulative effects of many genes which blend the effects of environmental factors (Lie, 1990; Hutt, 1974). In this thesis, the research focus was placed on demonstrating variation in the immune competence of pigs derived from different breed populations, with a view to determining whether variation in immune competence was present for pigs bred in Vietnam and Australia and to determine if an indigenous pig breed (Mong Cai) has a greater immune responsiveness than exotic pigs such as Yorkshire and Landrace. Comparative studies on the immune competence of small populations of pigs of various breeds were conducted.

The results of the present study clearly demonstrate the existence of variation between boars in immune competence of their offspring and also suggest breed differnces may also exist.

In the Vietnam study the indigenous Mong Cai pigs convincingly showed improved immune competence responsiveness relatively to the other two exotic breeds, Yorkshire and Landrace while in the study carried out in Brisbane, Australia, Large White pigs appeared to be superior to the Duroc pigs.

Vietnam and Brisbane represent two distinctly different regions in many geographical and environmental aspects. Whether these factors played a role in the variation observed in the study remains to be solved. As significant sire effects were present in most breed populations used in the study, further investigation into selection for superior sires is warranted. Jensen and Christensen (1981) have previously reported significant sex effects on immune competence in pig breeds similar to those in this study, the present investigation failed to indicate any difference arising from this factor. However, the dam effect on genetic variation in the immune competence of the pigs used in this study is not clear and requires further investigation.

The breeds and sires of high immune responsiveness as identified in this study require further studies on their heritability estimates, especially for variation within a breed, and the correlation with level of disease resistance before

assessment can be made on the usefulness of these immunological traits for any breeding programs. Nevertheless, the promising results from the study of the Mong Cai breed has delivered the practical value of exploring indigenous breeds for breeding disease resistant pigs for our future.