RNA-seq transcriptome profiling of porcine lung from two pig breeds in response to *Mycoplasma hyopneumoniae* infection

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**ABSTRACT**

**Background.** *Mycoplasma hyopneumoniae* (Mhp) is the main pathogen causing respiratory disease in the swine industry. Mhp infection rates differ across pig breeds, with Chinese native pig breeds that exhibit high fecundity (e.g., Jiangquhai, Meishan, Erhualian) more sensitive than Duroc, Landrace, and other imported pig breeds. However, the genetic basis of the immune response to Mhp infection in different pig breeds is largely unknown.

**Aims.** The aims of this study were to determine the relative Mhp susceptibility of the Chinese native Jiangquhai breed compared to the Duroc breed, and identify molecular mechanisms of differentially expressed genes (DEGs) using an RNA-sequencing (RNA-seq) approach.

**Methods.** Jiangquhai and Duroc pigs were artificially infected with the same Mhp dose. The entire experiment lasted 28 days. Daily weight gain, Mhp-specific antibody levels, and lung lesion scores were measured to evaluate the Mhp infection susceptibility of different breeds. Experimental pigs were slaughtered on the 28th day. Lung tissues were collected for total RNA extraction. RNA-seq was performed to identify DEGs, which were enriched by gene ontology (GO) and the Kyoto Encyclopedia annotation of Genes and Genomes (KEGG) databases. DEGs were validated with real-time quantitative polymerase chain reaction (RT-qPCR).

**Results.** Infection with the same Mhp dose produced a more serious condition in Jiangquhai pigs than in Duroc pigs. Jiangquhai pigs showed poorer growth, higher Mhp antibody levels, and more serious lung lesions compared with Duroc pigs. RNA-seq identified 2,250 and 3,526 DEGs in lung tissue from Jiangquhai and Duroc pigs, respectively. The two breeds shared 1,669 DEGs, which were involved in immune-relevant pathways including cytokine-cytokine receptor interaction, PI3K-Akt signaling pathway, and chemokine signaling pathway. Compared to Jiangquhai pigs, more chemokines, interferon response factors, and interleukins were specifically activated in Duroc pigs; *CXCL10*, *CCL4*, *IL6* and *IFNG* genes were significantly up-regulated, which may help Duroc pigs enhance immune response and reduce Mhp susceptibility.
Conclusion. This study demonstrated differential immune-related DEGs in lung tissue from the two breeds, and revealed an important role of genetics in the immune response to Mhp infection. The biological functions of these important DEGs should be further confirmed and maybe applied as molecular markers that improve pig health.

Subjects Biotechnology, Genomics, Veterinary Medicine
Keywords RNA-seq, Jiangquhai pig, Duroc pig, Mycoplasma hyopneumoniae, Candidate gene

INTRODUCTION

Mycoplasma hyopneumoniae (Mhp) exists in every country where pigs are raised and is the main pathogen leading to respiratory disease in the swine industry (Maes et al., 2008; Stark, Nicolet & Frey, 1998). The pathogen resides in the respiratory tract, and its secretions can be found in infected pigs for a long time (Maes et al., 1996). The main clinical symptoms of infected pigs are dry cough, as well as dramatically reduced porcine growth and feed conversion rates, which cause great losses to the pig industry (Maes et al., 1996; Sarradell et al., 2003).

Production practices on some Chinese pig farms revealed that Chinese local breeds are more sensitive to Mhp than imported breeds such as Duroc and Landrace. The Meishan and Erhualian, which are characterized by high fecundity, show extremely high susceptibility to Mhp infection (Fang et al., 2015; Maingi et al., 2014). This suggests that genetic components contribute to breed susceptibility or resistance to Mhp infection. Recently, it was reported that quantitative trait loci (QTLs) are associated with respiratory disease lesions, and five QTL were detected in Landrace pigs (Okamura et al., 2012). In Chinese Erhualian pigs, QTLs affecting respiratory disease were identified by a genome-wide association study; CXCL6, CXCL8, KIT, and CTBP2 were highlighted as candidates that might associated with resistance or susceptibility to swine enzootic pneumonia-like respiratory disease (Huang et al., 2016). However, the genetic basis for the immune response to Mhp infection among different pig breeds remains largely unknown.

Jiangquhai is a Chinese pig breed distributed in the central Jiangsu Province. Similar to the Meishan and Erhualian breeds, Jiangquhai pigs exhibit sensitivity to Mhp infection (Maingi et al., 2014). Vaccines and antibiotics are used to control the occurrence of mycoplasma pneumonia of swine (MPS); however, these methods are not sufficient. It is therefore necessary to study the molecular mechanism of pathogenesis. This knowledge can be applied to carry out disease resistance breeding. In this study, we investigated the immune response of Jiangquhai and Duroc pigs to artificial Mhp infection using an RNA-sequencing (RNA-seq) approach. The goals were to identify genetic components that contribute to Mhp susceptibility or resistance and lay a foundation for genetic breeding that improve pig health.
MATERIALS & METHODS

Experimental design and sample collection
Twenty healthy 50-day-old Jiangquhai pigs were selected from the Jiangquhai Pig Breed Conservation Farm (Taizhou, China), and twenty healthy 50-day-old Duroc pigs were selected from the Xingtai Agriculture and Animal Husbandry Technology Development Company (Yangzhou, China). All pigs were free of all major porcine diseases and confirmed to be negative for Mhp, PRRSV, pseudorabies virus, and classical swine fever virus infection by PCR or reverse-transcription (RT)-PCR. Jiangquhai and Duroc pigs were randomly assigned to the control or infected group during the experiment and raised separately in isolation. Ten Jiangquhai and ten Duroc pigs were inoculated with 5 mL viral suspension of a virulent strain of Mhp (106 colour changing units [CCU]) (Xiong et al., 2014), which was provided by the Veterinary Medicine Institute of Jiangsu Academy of Agricultural Sciences (Nanjing, China). The remaining ten Jiangquhai and ten Duroc pigs were treated with an equivalent volume of aseptic physiological saline, which served as a negative control group. Four groups (i.e., Jiangquhai infection, Jiangquhai control, Duroc infection, Duroc control) were raised in isolation rooms to prevent cross-infection. Approval for the study was provided by the ethics committee of Yangzhou University (SYXK(Su) IACUC 2016-0131).

On day 28, all pigs were euthanized by stunning, and lung tissues were collected and stored at −70 °C. At this time, pulmonary MPS lesions were confirmed and assessed with the scoring system (Steinmann, Blaha & Meemken, 2014; Lee et al., 2011). From the beginning to the end (day 28) of the study, all experimental pigs were weighed prior to feeding in the morning to compare weight gain between groups. To assess the immune response, blood samples were collected on days 0 and 28 via jugular venipuncture into normal serum tubes without anticoagulant. The serum was separated by centrifugation (1,600× g for 10 min at 4 °C), divided into aliquots, and stored at −20 °C until analysis. Mhp-specific antibody in peripheral blood was detected.

RNA-seq library preparation and sequencing
Total RNA was extracted from lung tissue of three infected and three control pigs of each breed (three pigs selected randomly from each group) using TRIzol reagent (Invitrogen, South San Francisco, CA, USA) following the manufacturer’s protocol. RNA integrity was evaluated using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Samples with an RNA Integrity Number (RIN) >7 were subjected to subsequent analysis. The libraries were constructed using the TruSeq Stranded mRNA LTSample Prep kit (Illumina, San Diego, CA, USA) according to the manufacturer’s instructions. Then, these libraries were sequenced on the Illumina Hiseq 2500 platform (Shanghai OE Biotech Co., Ltd, Shanghai, China), and 125-bp paired-end reads were generated.

Quality control and mapping
Raw data (raw reads) were processed using the NGS QC Toolkit (Ravi, Mukesh & Liu, 2012). Low-quality reads and those containing poly-N were removed to ensure high-quality mapping. Then the clean reads were mapped using the Sus scrofa genome 11.1
as a reference with bowtie2 (Langmead & Salzberg, 2012) or Tophat software packages (http://tophat.cbcb.umd.edu/) (Kim et al., 2013).

**RNA-seq data analysis**

The FPKM (Fragments Per Kilobase of transcript per Million fragments mapped) value of each gene was calculated using cufflinks (Cole et al., 2012), and the read counts of each gene were obtained by HTSeq-Count (Anders, Pyl & Huber, 2015). Differentially expressed genes (DEGs) were identified using the DESeq estimateSizeFactors and nbinomTest functions, corrected p-value of 0.05 and log2 (Fold change) (log2 FC) of 1 were set as the threshold for significantly differential expression. Hierarchical clustering analysis of DEGs was performed to inspect sample relations. The DEGs were annotated by GO functional enrichment and KEGG pathway analysis using the Database for Annotation, Visualization and Integrated Discovery (DAVID).

**RT-qPCR verification**

RNA was extracted from lung tissue of six infected and six control pigs of each breed using the TRIzol reagent following the manufacturer’s protocol. First-strand cDNA synthesis was performed using 5 µg of RNA and the Superscript II cDNA amplification system (Invitrogen, South San Francisco, CA, USA) according to the manufacturer’s protocol. Quantitative PCR was performed using an ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA) and Power SYBR Green PCR Master Mix (Invitrogen, South San Francisco, CA, USA). The gene for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was included as an endogenous control, and the specific primers used in the RT-qPCR assays are listed in Table S1. Relative expression of target genes was determined by the comparative cycle threshold (C_T) method (Livak & Schmittgen, 2001) and the ΔC_T value was calculated by subtracting the target C_T of each sample from its GAPDH C_T value.

**Statistical analysis**

Weight gain and antibody level data are presented as mean ± standard error (SE). Comparison of variables was performed using one-way analysis of variance with SPSS13.0 software (SPSS Inc., Chicago, IL, USA).

**RESULTS**

**Effect of Mhp infection on weight gain, antibody production, and lung lesions in Jiangquhai and Duroc pigs**

The average daily weight gain (ADG) of the Jiangquhai infected pigs was highly significantly lower than the Jiangquhai control pigs (p < 0.01), while the ADG of Duroc infected pigs was not significantly different from Duroc control pigs (p > 0.05) (Table 1). These results demonstrate that Mhp infection had a greater impact on the growth rate of Jiangquhai pigs compared to Duroc pigs.

On day 28, the level of Mhp-specific antibody (reported as the sample mean/positive control mean (s/p) value) in the peripheral blood of Jiangquhai infected pigs reached at 0.85 ± 0.20, which was significantly higher than that of Duroc infected pigs (0.48 ± 0.19) (p < 0.01) (Table 1). Mhp-specific antibody was not detected in control pigs.
Table 1  Average daily weight gain and Mhp-specific antibody levels of experimental pigs.

| Groups          | N  | Average body weight (kg) | ADG (g/d)         | Antibody levels (s/p) |
|-----------------|----|--------------------------|-------------------|-----------------------|
|                 |    | 0d                        | 28d               | 0d                    | 28d                   |
| Jiangquhai infection | 10 | 8.54 ± 0.77               | 12.26 ± 0.51      | 132.86 ± 14.56<sup>A</sup> | 0.05 ± 0.03          | 0.85 ± 0.20<sup>A</sup> |
| Jiangquhai control | 10 | 8.46 ± 0.50               | 15.29 ± 0.76      | 244.11 ± 21.10<sup>B</sup> | 0.05 ± 0.04          | 0.08 ± 0.07           |
| Duroc infection  | 10 | 10.64 ± 0.83              | 18.41 ± 0.49      | 277.50 ± 25.81        | 0.02 ± 0.01          | 0.48 ± 0.19<sup>B</sup> |
| Duroc control   | 10 | 10.53 ± 0.72              | 18.81 ± 0.58      | 295.89 ± 18.86        | 0.02 ± 0.01          | 0.04 ± 0.01           |

Notes.
Different letters in the same column indicate significant differences of mean values (A and B) (p < 0.01).

Our analysis of lung tissue on day 28 revealed that Jiangquhai infected pigs had more serious Mhp lung lesions (Fig. 1), a greater difference in Mhp lung lesion scores from Jiangquhai infected pigs compared to Duroc infected pigs (p < 0.01) (Fig. 2).

Figure 1  Lung tissue of experimental pigs. (A) Jiangquhai infected pigs, (B) Jiangquhai control pigs, (C) Duroc infected pigs, (D) Duroc control pigs. Lung pathological tissues are indicated with arrows.
Preliminary analysis and summary of RNA-Seq data quality

We performed RNA-seq to analyze the transcriptional profile of lung tissue from the four experimental groups. We found that 87.54–96.47% of clean reads were mapped to the reference genome (*Sus scrofa* 11.1), and 80.19–93.33% were uniquely mapped (Table S2). The sample to sample distance heat map showed a good degree of similarity between all three replicates (Fig. S1), indicating that there were no significant differences in gene expression among the biological replicates. These results showed that the RNA-seq data were reliable and met the conditions for differential expression analysis.

DEGs analysis and RT-qPCR validation

We analyzed the DEG profiles of lung tissues from Mhp-infected Jiangquhai and Duroc pigs by comparing infected and control animals of the same breed. Genes with relative expression levels that showed log2 FC > 1 (*p* < 0.05) were considered up-regulated, and those with log2 FC < −1 (*p* < 0.05) were considered down-regulated. Of the 2,250 DEGs detected in Jiangquhai pigs, 966 genes were up-regulated and 1,284 down-regulated. Of the 3,526 DEGs detected in Duroc pigs, 1,326 and 2,200 were up- and down-regulated, respectively (Fig. 3). Jiangquhai and Duroc pigs shared 1,669 DEGs in response to Mhp infection, with 632 up-regulated genes and 1,037 down-regulated genes. Further analysis showed that Duroc pigs had 694 uniquely up-regulated DEGs, which was two times higher than the number in Jiangquhai pigs (334 DEGs) (Fig. 3).

To verify the RNA-seq results, eight genes were randomly selected for RT-qPCR analysis. Expression FCs determined by RT-qPCR analysis was compared against the profiles predicted by RNA-seq. The RT-qPCR results verified the changes in expression levels of the eight genes (Fig. 4), indicating that the RNA-seq data were reliable.
Terms and pathways associated with immunobiology were enriched in both breeds

To determine the biological function of DEGs after Mhp infection in lung tissue, the common 1,669 DEGs were submitted to DAVID for GO analysis, which revealed enrichment of 281 GO terms ($p < 0.05$). The top 10 significant enrichments are shown in Fig. 5, among which cell adhesion, inflammatory response and immune response were the most significantly regulated by Mhp infection. Pathway analysis based on the KEGG database revealed the top 20 significant signaling pathways of the two breeds (Fig. 6). Many immune-related pathways were enriched, including the cytokine-cytokine receptor
Figure 5  The top 10 gene ontology (GO) enrichments of common DEGs shared by Jiangquhai and Duroc pigs. The Y-axis is the name of each category, the X-axis is their \(-\log_{10}(p\text{-value})\). 

interaction (\textit{IL18}, \textit{IL1R1}), PI3K-Akt signaling pathway (\textit{TLR2}, \textit{ITGA1}, \textit{ITGB7}, \textit{PIK3R5}), and chemokine signaling pathway (\textit{CXCL8}, \textit{CXCL13}).

**Specific terms and pathways in Jiangquhai and Duroc pigs**

Jiangquhai and Duroc pigs also had different responses to Mhp infection. The specific DEGs of Jiangquhai (581 DEGs) and Duroc pigs (1,857 DEGs) were separately submitted to DAVID for GO analysis. The results revealed 100 GO enrichments in Jiangquhai pigs and 314 GO enrichments in Duroc pigs. Table S3 shows the top 10 significant enrichments in Jiangquhai pigs, including cell adhesion, leukocyte migration and extracellular matrix disassembly. Table S4 lists the top 10 significant enrichments in Duroc pigs, including cilium assembly, cilium-dependent cell motility and interleukin-7-mediated signaling pathway.

KEGG pathway analysis revealed 38 specific signaling pathways in Jiangquhai pigs (Table S5) and 54 specific signaling pathways in Duroc pigs (Table S6). Further analysis found that Duroc pigs had more immune-related pathways (18 immune-related pathways), including chemokine signaling pathway, NOD-like receptor signaling pathway, antigen processing and presentation, toll-like receptor signaling pathway, 253 DEGs were enriched
in those 18 pathways (Table 2). Jiangquhai pigs only identified 8 immune-related pathways, including leukocyte transendothelial migration, hematopoietic cell lineage, cell adhesion molecules, 99 DEGs were enriched in those 8 pathways (Table 2).

**Analysis of important DEGs related to immune responses**

In this study, we mainly focused on the important DEGs related to immune responses. Based on gene clustering and specific KEGG pathways (Table 2), some DEGs that we identified were well-known components of the innate immune response, such as chemokines, interleukins, interferon response factors and complement components. Forty-five important specific DEGs related to innate immune responses had been identified in Duroc pigs (Fig. 7B), and only 20 important specific DEGs had been identified in Jiangquhai pigs (Fig. 7A). Furthermore, some immune-related genes were down-regulated in Jiangquhai pigs, such as adhesion molecules (*PECAM1, CD34, CD7, CDH4*), WNT molecules...
| Breeds     | Pathway terms                                    | Categories              | Genes                                                                                   |
|-----------|--------------------------------------------------|-------------------------|-----------------------------------------------------------------------------------------|
| Jiangquhai pigs | Cytosolic DNA-sensing pathway                     | Immune system           | IFNB1, NFKBIA, CXCL10, IRF7, ZBP1, CCL5, CCL4, DDX58, IL6, RIPK3, POLR3D, NFKBIB      |
|           | Intestinal immune network for IgA production     | Immune system           | CCR8, CXCL12, ICOS, SLA-DRB1, CD28, LOC106504372, IL10, IL6, AICDA, IL15RA              |
|           | Complement and coagulation cascades               | Immune system           | CIQA, C1S, F7, BDKRB1, F2, THBD, VSIG4, CIQ8, C1QC, VWF, SERPINE1, C7, CPB2, C1R       |
|           | Chemokine signaling pathway                      | Immune system           | CCR5, CCR1, XCR1, CXCR6, CCR9, CXCL12, NFKBIA, CXCL10, CXCL12, CCL5, AKT3, CCL4, PTK2B, ADcy4, GRK5, JAK3, CXCR3, NFKBIB, LOC110255211, GNG3, PARC, CXC2, HCK, ADCT1, PLCB2 |
|           | NOD-like receptor signaling pathway               | Immune system           | NFKBIA, CCL5, PSTITP1, TNFAIP3, IL6, MAPK10, TRIP6, NFKBIB, BIRC3, MAPK11              |
|           | RIG-I-like receptor signaling pathway             | Immune system           | IFNB1, NFKBIA, CXCL10, IRF7, ISG13, DDX58, DDX58, TRFC, MAPK10, NFKBIB, TANK, MAPK11 |
|           | Antigen processing and presentation               | Immune system           | TAP1, SLA-DRB1, CALR, PDA53, LOC106504372, CD8B, HSP7072, IFNG, PSME2, KLRD1, LOC100525789 |
|           | Toll-like receptor signaling pathway              | Immune system           | IFNB1, NFKBIA, CXCL10, IRF7, CD14, LY96, CCL5, IFNAR2, AKT3, CCL4, IL6, MAPK10, MAPK86, MAPK58, MAPK26, MAPK11 |
|           | T cell receptor signaling pathway                 | Immune system           | NFKBIA, ICOS, PDCD1, AKT3, CD28, CD8B, IFNG, IL10, CTLA4, NFKBIB, MAPK8, CARD11, MAPK11, PAK6, NFKBIE |
|           | TNF signaling pathway                             | Signal transduction     | CXCL2, NFKBIA, CXCL10, CREBSL1, CCL5, AKT3, CXCL10, TNFAIP3, FAS, PTG52, IL6, LTA, RIPK3, MAPK10, MAPR3, MAPK86, BIRC3, TRAF1, MAPK11 |
|           | Jak-STAT signaling pathway                        | Signal transduction     | IFNB1, IL17, SOCS1, IFNAR2, IL27RA, AKT3, IL2RA, C5P, IFNG, IL10, IL28G, IL6, JAK3, OSMB, IL15RA, IL20RB, LOC100573818, PIM1, CDK13NA, IL28B, TSLP, IL2, PTPN2 |
|           | cAMP signaling pathway                            | Signal transduction     | NFKBIA, TNX1, CREBSL1, GABBR1, SSTR1, PDI1, AKT3, HTTR1, AMH, ADcy4, MAPK10, GRI4A, GRI6A, PDE4D, SCRN1, LOC110259458, GRIN3A, PP1R18B, HCGR1, GRI1, ADCT1, GL1, PDE3B, GRIN2A, GRIN2B, CACNA1C, GIPR, CNGB1 |
|           | NF-kappa B signaling pathway                      | Signal transduction     | NFKBIA, CXCL12, CD14, LY96, BCL2A1, TNFAIP3, CCL4, DDX58, PTG52, LTA, BIRC3, TRAF1, NFKB2, CARD11 |
|           | MAPK signaling pathway                            | Signal transduction     | CD14, NTF3, DDIT3, PLAX4B, RP55EKA1, AKT3, HSP7072, FAS, EGF, FGFR4, NTRK1, FGFR4, MAPK10, DUSP4, PRKCG, GADD45B, MAPK86, PTPN7, CACNA2D2, CACNA2D3, FGFR2, CACNA1L, DUSP2, MAPK84, MAPK11, CACNA1C, CACNA1H, MAPK1, CACNA1E |
| Duroc pigs | FoxO signaling pathway                            | Signal transduction     | CCNB2, IL37, PCK2, CCNB1, AKT3, EGF, IL10, IL6, MAPK30, PKRAG2, GADD45B, CCNB3, CDEN1A, IRS2, PIK1, MAPK11 |
|           | Cytokine-cytokine receptor interaction            | Signaling molecules and interaction | CCR5, CCR1, XCR1, CXCR6, CCR9, CXCL2, IFNB1, CXCL10, CXCL12, CCL5, IL7R, IFNAR2, TNFRSF8, CD277, CCL4, IL2RA, FAS, C5P, IFNG, EGF, IL10, IL28G, AMH, IL6, LTA, OSMB, CPB3, CXCR4, IL15RA, IL20RB, LOC100573818, IL18RAP, IL29, IL28B, CXCR2, TSLP, IL22 |
|           | Cell adhesion molecules (CAMs)                    | Signaling molecules and interaction | ICOS, SELL, SLA-DRB1, OCLN, PDCD1, VCAN, CD1D1, CD28, LOC106504372, CD8B, CTLA4, MPZ, LTAM, TIGIT, NELGN3, LOC100525346, ITG8A, NCAIM2, NEGR1 |
|           | Neuroactive ligand–receptor interaction           | Signaling molecules and interaction | P2RY2, BDKRB1, F2, GABBR1, GZMA, CRHR2, SSTR1, P2RX1, TRH, TRPS1, HRH4, CRHR1, HRH1B, PTH1R, TAAR1, LTBR2, TRBP, FGFRB, GRI9A, P2RX3, PTGR1, GRIK1, GABBR2, CNR1, LRR7C4B, PARD3, GRIN3A, P2RX1, GRM2, GABBR3, GRIK2, GRI9A, GRIN2A, GRIN2B, GIPR |
|           | Complement and coagulation cascades               | Immune system           | CS, MBL1, C6, PLAU, F5, SERPINA5, ITGAM, KNG1, SERPINF2                                |
|           | Leukocyte transendothelial migration              | Immune system           | CLDN23, CLDN18, CLDN5, PECAM1, ITGAM, TXR, ESAM, GNA1H, PLCG2                        |
|           | Natural killer cell mediated cytotoxicity         | Immune system           | NC1R1, IFNAR1, KLRC1, CD244, LOC1005739680, SHC3, LOC100525789, PLCG2                  |
|           | Hematopoietic cell lineage                        | Immune system           | CD4, GP1BB, CD34, CD19, ITGAM, CD7                                                   |
|           | Hippo signaling pathway                           | Signal transduction     | PARD6B, BMP2, WNT10B, WNT16, GDF7, WNT11, TCF7L1, GDF6, WNT15, FZD1                   |
|           | cAMP signaling pathway                            | Signal transduction     | FXYD2, FO2, PDE4A, DRD2, GNA1, LOC10037482, LOC110255845, LOC110255846, CACNA1F, PDE4C |
|           | Notch signaling pathway                           | Signal transduction     | HES1, DTX2, RBPIL                                                                  |
|           | Cell adhesion molecules (CAMs)                    | Signaling molecules and interaction | NECTIN1, CD4, CLDN23, CLDN18, CLDN5, PECAM1, CD4, ITGAM, VTCN1, ESAM, CDHA, CNTNAP2 |
Figure 7 Important immune-related genes showing specific expression in Jiangquhai pigs (A) and Duroc pigs (B). Genes have been arbitrarily positioned along the x-axis.

(WNT11, WNT16). As for Duroc pigs, more immune-related genes were up-regulated, including chemokines (CCL4, CCL5, CCR1, CCR5, CXCL2, CXCL10, CXCL12, CXCIR6), interferon response factors (IFNAR2, IFNG), and interleukins (IL6, IL10, IL15RA, IL2RA, IL18RAP, IL2RB, IL2RG, IL27RA, IL7R). Among these immune-related genes, CXCL10, CCL4, IL6 and IFNG were the most differently expressed genes.

**DISCUSSION**

Previous reports state that the primary effect of Mhp infection on pigs is reduced growth performance (Maes et al., 2008; Pointon, Byrt & Heap, 1985). However, the degree to which Mhp effect differs between Chinese local breeds and imported breeds (such as Duroc and Landrace), and the effect on Chinese local breeds is much more serious (Fang et al., 2015). Our results were consistent with previous reports, Jiangquhai pigs infected with Mhp exhibited poorer growth performance than Duroc pigs. Some studies have reported that Mhp infection causing a humoral immune response in pigs (Blanchard et al., 1992). Moreover, the level of antigen-specific antibody is an important indicator of MPS lesion...
formation in animals, and increased antigen-specific antibody production may exacerbate MPS lung lesion severity (Borjigin et al., 2016; Davis et al., 1985; Katayama et al., 2011). Our results also showed Mhp-infected Jiangquhai pigs exhibited higher blood levels of Mhp antibody and more serious Mhp lung lesions. These results confirm that Jiangquhai pigs are more susceptible to Mhp infection, and Duroc pigs possess greater resistance. In addition, it was reported that the age and weight of pigs do not affect the susceptibility to Mhp infection (Piffer & Ross, 1984). Therefore, it is theoretically possible that genetic components contribute to Mhp resistance/susceptibility differs among breeds.

To gain insight into how the transcriptome profiles of different pig breeds vary in response to Mhp infection, we performed RNA-seq to analyze the transcriptional profiles of lung tissue from two breeds. We identified 2,250 and 3,526 DEGs in lung tissue from Jiangquhai pigs and Duroc pigs, respectively. Duroc pigs had 694 unique up-regulated DEGs, whereas Jiangquhai pigs only had 334. Taken together, these results indicate that the molecular interactions and signaling pathways following Mhp infection may be more complex in Duroc pigs.

Our analysis revealed 1,669 DEGs shared between Jiangquhai and Duroc pigs in response to Mhp infection. These common DEGs also showed significant enrichment of many immune-relevant terms and pathways. Among these, many up-regulated immune-relevant genes were observed in both breeds that could play a role in resistance to Mhp infection. One TLR family member, TLR2, has a fundamental role in pathogen recognition, signal transmission and activation of innate immunity, and stimulation of inflammatory cytokine production (Yoshihiro et al., 2003). IL18 and IL1R1 are pro-inflammatory mediators involved in many cytokine-induced immune and inflammatory responses (Dale & Nicklin, 1999). PI3Ks (PIK3R5) are important enzymes involved in various signal transduction pathways, and play important roles in regulating cell growth, survival, death and chemotaxis (Margaria et al., 2019). Chemokines such as CXCL8 and CXCL13 can recruit immune cells to the site of infection (Strieter et al., 1999; Sun et al., 2006). Moreover, ITGA1 and ITGB7, which help recruit immune cells such as T, B, and natural killer (NK) cells, were up-regulated following Mhp infection (Campbell & Humphries, 2011; Cheli et al., 2007; Lim, Leung & Krissansen, 1998). These immune-related DEGs help clarify the immune response in pigs following Mhp infection.

Comparison of the specific DEGs between Jiangquhai and Duroc pigs revealed some important DEGs related to immune responses were specifically altered in Jiangquhai or Duroc pigs, and these important DEGs enriched in immune-related pathways play an important role in the underlying host mechanism to defend against Mhp infection.

Adhesion molecules are important signal transmitters of the immune system, they function as signal transduction, transported signals from outside to inside of cell (Kumar et al., 2016; Korytina et al., 2019). In this study, many adhesion molecules were specifically down-regulated in Jiangquhai pigs, including PECAM1, CD34, CD7 and CDH4. These adhesion molecules were significantly enriched in Cell adhesion molecules (CAMs) and Hematopoietic cell lineage pathway, have the function in pathogen recognition, signal transmission and regulating cell movement (Chasiotis et al., 2012; Samanta & Almo, 2015; Xiao et al., 2010). However, these adhesion molecules were down-regulated may reduce
host’s capacity for antigen presentation and processing. In addition, two WNT molecules (WNT11, WNT16) enriched in Hippo signaling pathway were identified in Jiangquhai pigs. WNT genes exert immune modulatory functions during pathogens infection (Brandenburg & Reiling, 2016), and could regulate the expression of immune response genes during challenge by pathogens, such as interferon genes, Toll-like receptors and MHC genes (Garcia-Rodriguez et al., 2017). WNT11 and WNT16 were down-regulated, which may induce the inhibition of its downstream signaling, including cell growth, survival and cell-cycle progression. Therefore, thus results may interfere with Jiangquhai pigs to establish an effective immune response against Mhp infection.

Chemokines constitute a large family of chemotactic molecules that are fundamentally involved in the inflammatory response by attracting immune cells to sites of inflammation, and promote the immune response and wound healing (Le et al., 2004; Zlotnik & Yoshie, 2012). Many chemokines are expressed in immune tissues and cells in pigs infected with Mhp (Li et al., 2014; Zhang et al., 2011). In this study, many specific up-regulated chemokines (CCL4, CCL5, CCR1, CCR5, CXCL2, CXCL10, CXCL12 and CXCR6) were identified in Duroc pigs, these chemokines were mainly enriched in cytokine-cytokine receptor interaction and chemokine signaling pathway. The two pathways are involved in specific functional tasks that recruit immune cells to induce inflammatory and adaptive immune responses (Carvalho et al., 2012; Hu et al., 2016). Therefore, these up-regulated chemokines could recruit more immune cells for pathogen defense, and Duroc pigs exhibited a larger chemotactic immune cell capacity. Among these chemokines, CXCL10 and CCL4 were most highly expressed. CXCL10 is an important regulator of pulmonary diseases (Gao et al., 2018; Tighe et al., 2011), and has the function of chemotactic monocytes/macrophages, T cells, and NK cells and promotion of T-cell adhesion to endothelial cells (Angiolillo et al., 1995; Dufour et al., 2002). CCL4 is an important chemoattractant for natural killer cells, monocytes and a variety of other immune cells, regulates immune response to pathogen infection (Bystry et al., 2001; Zhao et al., 2007). Therefore, CXCL10 and CCL4 maybe important chemokines involved in chemokine signaling pathway, and regulate the process of immune response to Mhp infection.

Mhp stimulates host immune response by inducing macrophages to release pro-inflammatory cytokines, such as IL-2, IL-6, IL-8, IL10 and IL-1β (Fourour et al., 2019; Lorenzo et al., 2006). These pro-inflammatory cytokines are important players in innate and adaptive immunity, and affect immune balance by suppressing cell-mediated immunity (Zhang et al., 2018). In this study, we also identified some specific up-regulated interleukins (IL6, IL10, IL15R A, IL2RA, IL18RAP, IL2RB, IL2RG, IL27RA and IL7R) in Duroc pigs. These interleukin cytokines can modulate a broad spectrum of immune response processes, such as NOD-like receptor signaling pathway, toll-like receptor signaling pathway, TNF signaling pathway and Jak-STAT signaling pathway. Thus, activation of interleukin cytokines in Duroc pigs may induce additional immune cytokine production and immune cell recruitment for pathogen defense. In addition, interferon response factors have the function of activate macrophages, protect host cells to resist pathogen infection (Rodriguez et al., 2007). Two interferon response factors (IFNAR2, IFNG) were highly expressed in Duroc pigs, which may further activated macrophages and enhanced the ability to resistance Mhp
infection. Moreover, \textit{IL6} and \textit{IFNG} were most highly expressed among these cytokines. \textit{IL-6} is a primary cytokine, which could activate macrophages to secrete inflammatory cytokines and chemokines (Yan et al., 2012), and responsible for Mhp clearance in lungs (Wu et al., 2008). \textit{IFNG} had been detected in the lungs of pathogen-infected pigs and be postulated to be a necessary component for host control of pathogen (Zhang et al., 2011). Therefore, \textit{IL6} and \textit{IFNG} were also important cytokines involved in the regulation of immune response to Mhp infection.

Apoptosis is a mechanism of programmed cell death and is essential for the regulation of immune responses (Rantong & Gunawardena, 2015). Our results further revealed that the apoptosis-related gene (\textit{FAS}) was specifically activated in Duroc pigs. The \textit{FAS} gene has been reported to play a central role in the physiological regulation of programmed cell death and has been implicated in the pathogenesis of various diseases of the immune system (Strasser, Jost & Nagata, 2009). Previous studies have shown that the \textit{FAS} gene involved in the regulation of inflammatory response in pulmonary (Liu et al., 2017). Therefore, the \textit{FAS} gene activation can induce apoptosis and regulate the immune response in response to the Mhp infection. In addition, in the Duroc-specific DEGs involved in GO enrichments (cilium assembly, cilium-dependent cell motility), we found intraflagellar transport (\textit{IFT}) genes, which can modulate primary cilia formation and function (Pedersen & Rosenbaum, 2008), such as \textit{IFT172} and \textit{IFT81}. Primary cilia are found on the cell surface of almost every cell type, which play an important role in signaling and development (Singla & Reiter, 2006) and influence immune cell migration (Finetti, Onnis & Baldari, 2015). Therefore, intraflagellar transport genes may play an important role in the immune response to Mhp infection. Further elucidating the function remains to be a near future goal.

**CONCLUSIONS**

This is the first study to describe the transcriptional profiles of lung tissue from different pig breeds following Mhp infection. RNA-seq analysis identified 966 up-regulated and 1,284 down-regulated genes in Jiangquhai pigs compared to 1,326 up-regulated and 2,200 down-regulated genes in Duroc pigs. Both breeds shared some KEGG pathways, including cytokine-cytokine receptor interaction, PI3K-Akt signaling pathway, and chemokine signaling pathway. All of these may play important roles in Mhp infection resistance. In Duroc pigs, 1857 specific DEGs were identified, KEGG pathway analysis revealed 18 immune-related pathways. In Jiangquhai pigs, 581 specific DEGs were identified and eight immune-related pathways were identified. Compared to Jiangquhai pigs, chemokines, interferon response factors, interleukins, complement components, apoptosis-related molecule and other immune-related molecules were specifically activated in Duroc pigs, and they may help host enhance immune response and reduce Mhp susceptibility. The results of our analysis reveal an important role of genetics in the immune response to Mhp infection, and this should be investigated further to improve pig health during breeding.
ADDITIONAL INFORMATION AND DECLARATIONS

Funding
This work was supported by the National Development and Reform Commission Special Breeding Projects (No. 2150299) and the Phoenix Talent Projects of Jiangsu Agri-animal Husbandry Vocational College (No. 201602). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures
The following grant information was disclosed by the authors:
National Development and Reform Commission Special Breeding Projects: 2150299.
Jiangsu Agri-animal Husbandry Vocational College: 201602.

Competing Interests
The authors declare there are no competing interests.

Author Contributions
• Ligang Ni conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables.
• Chengyi Song conceived and designed the experiments.
• Xinsheng Wu analyzed the data, authored or reviewed drafts of the paper, approved the final draft.
• Xuting Zhao analyzed the data.
• Xiaoyan Wang performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
• Bichun Li contributed reagents/materials/analysis tools.
• Yuan Gan performed the experiments.

Animal Ethics
The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):
Approval for the study was provided by the ethics committee of Yangzhou University (SYXX(Su) IACUC 2016–0131).

DNA Deposition
The following information was supplied regarding the deposition of DNA sequences:
The sequences are available at the NCBI Short Read Archive (SRA) under BioProject: PRJNA542576. Raw reads are available in the Sequence Read Archive database (SRR9114668–SRR9114679).

Data Availability
The following information was supplied regarding data availability:
The raw measurements are available in Datasets S1–S3.
Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.7900#supplemental-information.

REFERENCES

Anders S, Pyl PT, Huber W. 2015. HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics 31(2):166–169 DOI 10.1093/bioinformatics/btu638.

Angiolillo AL, Sgadari C, Taub DD, Liao F, Farber JM, Maheshwari S, Kleinman HK, Reaman GH, Tosato G. 1995. Human interferon-inducible protein 10 is a potent inhibitor of angiogenesis in vivo. Journal of Experimental Medicine 182(1):155–162 DOI 10.1084/jem.182.1.155.

Blanchard B, Vena MM, Cavalier A, Lannic JL, Gouranton J, Kobisch M. 1992. Electron microscopic observation of the respiratory tract of SPF piglets inoculated with Mycoplasma hyopneumoniae. Veterinary Microbiology 30(4):329–341 DOI 10.1016/0378-1135(92)90020-T.

Borjigin L, Shimazu T, Katayama Y, Li M, Satoh T, Watanabe K, Kitazawa H, Roh SG, Aso H, Katoh K. 2016. Immunogenic properties of Landrace pigs selected for resistance to mycoplasma pneumonia of swine. Animal Science Journal 87(3):321–329 DOI 10.1111/asj.12440.

Brandenburg J, Reiling N. 2016. The Wnt blows: on the functional role of Wnt signaling in mycobacterium tuberculosis infection and beyond. Frontiers in Immunology 7:Article 635 DOI 10.3389/fimmu.2016.00635.

Bystry RS, Aluvihare V, Welch KA, Kallikouri M, Betz AG. 2001. B cells and professional APCs recruit regulatory T cells via CCL4. Nature Immunology 2:1126–1132 DOI 10.1038/ni735.

Campbell I, Humphries M. 2011. Integrin structure, activation, and interactions. Cold Spring Harbor perspectives in biology 3(3):Article a004994 DOI 10.1101/cshperspect.a004994.

Carvalho FA, Nalbantoglu I, Aitken JD, Uchiyama R, Su Y, Doho GH, Vijay-Kumar M, Gewirtz AT. 2012. Cytosolic flagellin receptor NLRC4 protects mice against mucosal and systemic challenges. Mucosal Immunology 5(3):288–298 DOI 10.1038/mi.2012.8.

Chasiotis H, Kolosov D, Bui P, Kelly S. 2012. Tight junctions, tight junction proteins and paracellular permeability across the gill epithelium of fishes: a review. Respiratory Physiology & Neurobiology 184(3):269–281 DOI 10.1016/j.resp.2012.05.020.

Cheli Y, Kanaji S, Jacquelin B, Chang M, Nugent D, Kunicki T. 2007. Transcriptional and epigenetic regulation of the integrin collagen receptor locus ITGA1-PELO-ITGA2. Biochimica Et Biophysica Acta Gene Structure 1769(9–10):546–558 DOI 10.1016/j.bbaexp.2007.06.004.

Cole T, Adam R, Loyal G, Geo P, Daehwan K, Kelley DR, Harold P, Salzberg SL, Rinn JL, Lior P. 2012. Differential gene and transcript expression analysis of
RNA-seq experiments with TopHat and Cufflinks. *Nature Protocols* **7**(3):562–578 DOI 10.1038/nprot.2012.016.

Dale M, Nicklin MJ. 1999. Interleukin-1 receptor cluster: gene organization of IL1R2, IL1R1, IL1RL2 (IL-1Rrp2), IL1RL1 (T1/ST2), and IL1R1 (IL-1Rrp) on human chromosome 2q. *Genomics* **57**(1):177–179 DOI 10.1006/geno.1999.5767.

Davis JK, Parker RF, White H, Dziedzic D, Taylor G, Davidson MK, Cox NR, Cassell GH. 1985. Strain differences in susceptibility to murine respiratory mycoplasmosis in C57BL/6 and C3H/HeN mice. *Infection & Immunity* **50**(3):647–654 DOI 10.1016/0162-3109(85)90015-3.

Dufour JH, Michelle D, Liu MT, Leung JH, Lane TE, Luster AD. 2002. IFN-gamma-inducible protein 10 (IP-10; CXCL10)-deficient mice reveal a role for IP-10 in effector T cell generation and trafficking. *Journal of Immunology* **168**(7):3195–3204 DOI 10.4049/jimmunol.168.7.3195.

Fang X, Zhao W, Fu Y, Tu F, Li B, Wang X, Zhao F, Ren S. 2015. Difference in susceptibility to Mycoplasma pneumonia among various pig breeds and its molecular genetic basis. *Scientia Agricultura Sinica* **48**(14):2839–2847 DOI 10.3864/j.issn.0578-1752.2015.14.015.

Finetti F, Onnis A, Baldari CT. 2015. Regulation of vesicular traffic at the T cell immune synapse: lessons from the primary cilium. *Traffic* **16**(3):241–249 DOI 10.1111/tra.12241.

Fourour S, Marois-Crehan C, Martelelt L, Fablet C, Kempf I, Gottschalk M, Segura M. 2019. Intra-species and inter-species differences in cytokine production by porcine antigen-presenting cells stimulated by Mycoplasma hyopneumoniae, M. hyorhinis, and M. flocculare. *Pathogens* **8**(1):Article 34 DOI 10.3390/pathogens8010034.

Gao B, Lin J, Jiang Z, Yang Z, Yu H, Ding L, Yu M, Cui Q, Neil D, Zhang M, Li M. 2018. Upregulation of chemokine CXCL10 enhances chronic pulmonary inflammation in tree shrew collagen-induced arthritis. *Scientific Reports* **8**(1):Article 9993 DOI 10.1038/s41598-018-28404-y.

Garcia-Rodriguez K, Goenka A, Alonso-Rasgado M, Hernandez-Pando R, Bulfone-Paus S. 2017. The role of mast cells in tuberculosis: orchestrating innate immune crosstalk? *Frontiers in Immunology* **8**:Article 1290 DOI 10.3389/fimmu.2017.01290.

Hu JQ, Yang DD, Wang H, Li CH, Zeng YQ, Chen W. 2016. CpG oligodeoxynucleotides induce differential cytokine and chemokine gene expression profiles in dapulian and landrace pigs. *Frontiers in Microbiology* **7**:Article 1992 DOI 10.3389/fmicb.2016.01992.

Huang X, Huang T, Deng W, Yan G, Qiu H, Huang Y, Ke S, Hou Y, Zhang Y, Zhang Z. 2016. Genome-wide association studies identify susceptibility loci affecting respiratory disease in Chinese erhualian pigs under natural conditions. *Animal Genetics* **48**(1):30–37 DOI 10.1111/age.12504.

Katayama M, Fukuda T, Okamura T, Suda Y, Suzuki E, Uenishi H, Suzuki K. 2011. Immunophenotype characterization for swine selected line, which is resistant for the Mycoplasma pneumonia. *Asian Australasian Journal of Animal Sciences* **24**(7):889–897 DOI 10.5713/ajas.2011.10391.
Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. 2013. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biology* 14(4):Article R36 DOI 10.1186/gb-2013-14-4-r36.

Korytina GF, Akhmadishina LZ, Kochetova OV, Aznabaeva YG, Zagidullin SZ, Victorova TV. 2019. The role of serum amyloid A1, adhesion molecules, chemokines, and chemokine receptors genes in chronic obstructive pulmonary disease. *Russian Journal of Genetics* 55(1):105–113 DOI 10.1134/S1022795418120050.

Kumar A, Ouyang M, VandenDries K, McGhee EJ, Tanaka K, Anderson MD, Groisman A, Goult BT, Anderson KI, Schwartz MA. 2016. Talin tension sensor reveals novel features of focal adhesion force transmission and mechanosensitivity. *Journal of Cell Biology* 213(3):371–383 DOI 10.1083/jcb.20151001207062016c.

Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. *Nature Methods* 9(4):357–359 DOI 10.1038/nmeth.1923.

Le Y, Zhou Y, Iribarren P, Wang J. 2004. Chemokines and chemokine receptors: their manifold roles in homeostasis and disease. *Cellular & Molecular Immunology* 1(2):95–104.

Lee CH, Hwang WM, Lee JG, Lee SM, Kim SJ, Kim NH, Yang DS, Han JH. 2011. Study on gross finding of lung lesions and causative pathogens of porcine respiratory disease complex from slaughtered pigs in Incheon. *Journal of Applied Physics* 34(4):313–320 DOI 10.7853/kjvs.2011.34.4.313.

Li B, Du L, Sun B, Yu Z, Liu M, Feng Z, Wei Y, Wang H, Shao G, He K. 2014. Transcription analysis of the porcine alveolar macrophage response to Mycoplasma hyopneumoniae. *PLOS ONE* 9(8):Article e101968 DOI 10.1371/journal.pone.0101968.

Lim S, Leung E, Krissansen G. 1998. The beta7 integrin gene (Itgb-7) promoter is responsive to TGF. *Immunogenetics* 48(3):184–195 DOI 10.1007/s002510050422.

Liu X, Wong SS, Taype CA, Kim J, Shentu TP, Espinoza CR, Finley JC, Bradley JE, Head BP, Patel HH, Mah EJ, Hagood JS. 2017. Thy-1 interaction with Fas in lipid rafts regulates fibroblast apoptosis and lung injury resolution. *Laboratory Investigation* 97(3):256–267 DOI 10.1038/labinvest.2016.145.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25(4):402–408 DOI 10.1006/meth.2001.1262.

Lorenzo H, Quesada Ó, Assuncao P, Castro A, Rodriguez F. 2006. Cytokine expression in porcine lungs experimentally infected with Mycoplasma hyopneumoniae. *Veterinary Immunology and Immunopathology* 109(3–4):199–207 DOI 10.1016/j.vetimm.2005.07.021.

Maes D, Segales J, Meyns T, Sibila M, Pieters M, Haesebrouck F. 2008. Control of mycoplasma hyopneumoniae infections in pigs. *Veterinary Microbiology* 126(4):297–309 DOI 10.1016/j.vetmic.2007.09.008.

Maes D, Verdonck M, Deluyker H, De Kruif A. 1996. Enzootic pneumonia in pigs. *Veterinary Quarterly* 18(3):104–109 DOI 10.1017/s002217240002163x.

Maingi JW, Xiong QY, Wei YN, Ma QH, Ji Y, Kimaru W, Hua LZ, Wang J, Shao GQ, Bao ED. 2014. Detection of respiratory pathogens Mycoplasma hyorhinis...
and Mycoplasma hyopneumoniae from clinically infected porcine using nested PCR in Jiangsu Province, China. Chinese Journal of Zoonoses 30(8):800–805 DOI 10.3969/cjz.jssn.1002-2694.2014.08.006.

Margaria JP, Ratto E, Gozzelino L, Li H, Hirsch E. 2019. Class II PI3Ks at the intersection between signal transduction and membrane trafficking. Biomolecules 9(3):Article 104 DOI 10.3390/biom9030104.

Okamura T, Onodera W, Tayama T, Kadowaki H, Kojima-Shibata C, Suzuki E, Uemoto Y, Mikawa S, Hayashi T, Awata T. 2012. A genome-wide scan for quantitative trait loci affecting respiratory disease and immune capacity in Landrace pigs. Animal Genetics 43(6):721–729 DOI 10.1111/j.1365-2052.2012.02359.x.

Pedersen LB, Rosenbaum JL. 2008. Intraflagellar transport (IFT) role in ciliary assembly, resorption and signalling. Current Topics in Developmental Biology 85:23–61 DOI 10.1016/S0070-2153(08)00802-8.

Piffer IA, Ross RF. 1984. Effect of age on susceptibility of pigs to Mycoplasma hyopneumoniae pneumonia. American Journal of Veterinary Research 45(3):478–481 DOI 10.1136/vr.114.13.327-a.

Pointon AM, Byrt D, Heap P. 1985. Effect of enzootic pneumonia of pigs on growth performance. Australian Veterinary Journal 62(1):13–18 DOI 10.1111/j.1751-0813.1985.tb06032.x.

Rantong G, Gunawardena A. 2015. Programmed cell death: genes involved in signaling, regulation, and execution in plants and animals. Botany 93(4):193–210 DOI 10.1139/cjb-2014-0152.

Ravi K, Mukesh J, Liu Z. 2012. NGS QC Toolkit: a toolkit for quality control of next generation sequencing data. PLOS ONE 7(2):e30619 DOI 10.1371/journal.pone.0030619.

Rodriguez F, Quesada O, Poveda J, Fernandez A, Lorenzo H. 2007. Immunohistochemical detection of interleukin-12 and interferon-γ in pigs experimentally infected with Mycoplasma hyopneumoniae. Journal of Comparative Pathology 136(1):79–82 DOI 10.1016/j.jcpath.2006.11.001.

Samanta D, Almo SC. 2015. Nectin family of cell-adhesion molecules: structural and molecular aspects of function and specificity. Cellular and Molecular Life Sciences 72(4):645–658 DOI 10.1007/s00018-014-1763-4.

Sarradell J, Andrada M, Ramirez AS, Fernández A, Gómez-Villamandos JC, Jover A, Lorenzo H, Herráez P, Rodríguez F. 2003. A morphologic and immunohistochemical study of the bronchus-associated lymphoid tissue of pigs naturally infected with mycoplasma hyopneumoniae. Veterinary Pathology 40(4):395–404 DOI 10.1354/vp.40-4-395.

Singla V, Reiter JF. 2006. The primary cilium as the cell’s antenna: signaling at a sensory organelle. Science 313(5787):629–633 DOI 10.1126/science.1124534.

Stark KD, Nicolet J, Frey J. 1998. Detection of mycoplasma hyopneumoniae by air sampling with a nested PCR assay. Applied & Environmental Microbiology 64(2):543–548 DOI 10.1016/j.neucom.2004.10.080.

Steinmann T, Blaha T, Meemken D. 2014. A simplified evaluation system of surface-related lung lesions of pigs for official meat inspection under industrial...
slaughter conditions in Germany. *BMC Veterinary Research* **10**(1):Article 98 DOI 10.1186/1746-6148-10-98.

**Strasser A, Jost PJ, Nagata S. 2009.** The many roles of FAS receptor signaling in the immune system. *Immunity* **30**(2):180–192 DOI 10.1016/j.immuni.2009.01.001.

**Strieter RM, Kunkel SL, Keane MP, Standiford TJ. 1999.** Chemokines in lung injury: Thomas A. Neff Lecture. *Chest* **116**(1 Suppl):103S–110S DOI 10.1378/chest.116.suppl_1.103S.

**Sun X, Jones HP, Hodge LM, Simecka JW. 2006.** Cytokine and chemokine transcription profile during Mycoplasma pulmonis infection in susceptible and resistant strains of mice: macrophage inflammatory protein 1beta (CCL4) and monocyte chemoattractant protein 2 (CCL8) and accumulation of CCR5+ Th cells. *Infection & Immunity* **74**(10):5943–5954 DOI 10.1128/IAI.00082-06.

**Tighe RM, Liang J, Liu N, Jung Y, Jiang D, Gunn MD, Noble PW. 2011.** Recruited exudative macrophages selectively produce CXCL10 after noninfectious lung injury. *American Journal of Respiratory Cell & Molecular Biology* **45**(4):781–788 DOI 10.1165/rcmb.2010-0471OC.

**Wu Q, Martin RJ, Lafasto S, Efaw BJ, Rino JG, Harbeck RJ, Chu HW. 2008.** Toll-like receptor 2 down-regulation in established mouse allergic lungs contributes to decreased mycoplasma clearance. *American Journal of Respiratory & Critical Care Medicine* **177**(7):720–729 DOI 10.1164/rccm.200709-1387OC.

**Xiao SQ, Mo DW, Wang QW, Jia JY, Qin LM, Yu XC, Niu YN, Zhao X, Liu XH. 2010.** Aberrant host immune response induced by highly virulent PRRSV identified by digital gene expression tag profiling. *BMC Genomics* **11**(1):544 DOI 10.1186/1471-2164-11-544.

**Xiong QY, Wei YN, Feng ZX, Gan Y, Liu ZJ, Liu MJ, Bai FF, Shao GQ. 2014.** Protective efficacy of a live attenuated Mycoplasma hyopneumoniae vaccine with an ISCOM-matrix adjuvant in pigs. *Veterinary Journal* **199**(2):268–274 DOI 10.1016/j.tvjl.2013.11.001.

**Yan C, Wu M, Cao J, Tang H, Zhu M, Johnson P, Gao H. 2012.** Critical role for CCAAT/enhancer-binding protein β in immune complex-induced acute lung injury. *Journal of Immunology* **189**(3):1480–1490 DOI 10.4049/jimmunol.1200877.

**Yoshihiro M, Hirohide U, Reiko K, Kazuhiro Y, Yoshihiro S, Ryuji Y, Noriyuki H, Yuichi Y, Yasuyuki M. 2003.** Porcine TLR2 and TLR6: identification and their involvement in Mycoplasma hyopneumoniae infection. *Journal of Interferon & Cytokine Research* **23**(10):583–590 DOI 10.1089/107999003322485080.

**Zhang H, Lunney JK, Baker RB, Opriessnig T. 2011.** Cytokine and chemokine mRNA expression profiles in tracheobronchial lymph nodes from pigs singularly infected or coinfected with porcine circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (MHYO). *Veterinary Immunology and Immunopathology* **140**(1–2):152–158 DOI 10.1016/j.vetimm.2010.11.019.

**Zhang Z, Wei Y, Liu B, Wu Y, Wang H, Xie X, Feng Z, Shao G, Xiong Q. 2018.** Hsp90/Sec22b promotes unconventional secretion of mature-IL-1β through
an autophagosomal carrier in porcine alveolar macrophages during Mycoplasma hyopneumoniae infection. *Molecular Immunology* **101**:130–139 DOI 10.1016/j.molimm.2018.06.265.

Zhao W, Pahar B, Borda JT, Alvarez X, Sestak K. 2007. A decline in CCL3-5 chemokine gene expression during primary simian-human immunodeficiency virus infection. *PLOS ONE* **2**(8):e726 DOI 10.1371/journal.pone.0000726.

Zlotnik A, Yoshie O. 2012. The chemokine superfamily revisited. *Immunity* **36**(5):705–716 DOI 10.1016/j.immuni.2012.05.008.