Relational database models and other software and their importance in data analysis, storage, and communication

Eric Fritz
Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/etd
Part of the Animal Sciences Commons

Recommended Citation
Fritz, Eric, "Relational database models and other software and their importance in data analysis, storage, and communication" (2009).
Graduate Theses and Dissertations. 10849.
https://lib.dr.iastate.edu/etd/10849

This Thesis is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Graduate Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
Relational database models and other software and their importance in data analysis, storage, and communication

by

Eric Ryan Fritz

A thesis submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Animal Breeding and Genetics

Program of Study Committee:
James Reecy, Major Professor
Leslie Miller
Susan Lamont

Iowa State University
Ames, Iowa
2009

Copyright © Eric Ryan Fritz, 2009. All rights reserved.
# TABLE OF CONTENTS

ABSTRACT iv  
CHAPTER 1. GENERAL INTRODUCTION 1  
  AIMS AND OBJECTIVES 2  
  THESIS ORGANIZATION 2  
  REVIEW OF RELEVANT LITERATURE 3  
  DATABASES MODELS 3  
  OTHER COMPUTER SOFTWARE 10  
  REFERENCES 14  

CHAPTER 2. BEAP: THE BLAST EXTENSION AND ALIGNMENT PROGRAM- A TOOL FOR CONTIG CONSTRUCTION AND ANALYSIS OF PRELIMINARY GENOME SEQUENCE 16  
  ABSTRACT 16  
  FINDINGS 17  
  METHODS 17  
  RESULTS 21  
  DISCUSSION 23  
  CONCLUSIONS 24  
  ACKNOWLEDGEMENTS 25  
  REFERENCES 25  
  FIGURES 27  
  TABLES 27  

CHAPTER 3. THE EFFECT OF SWINE LEUKOCYTE ANTIGEN (SLA) GENES ON PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) IMMUNITY: VIRAL, SERUM NEUTRALIZING ANTIBODY, AND CYTOKINE LEVELS 29  
  ABSTRACT 30  
  INTRODUCTION 30  
  MATERIALS AND METHODS 32  
  RESULTS 37  
  DISCUSSION 43  
  REFERENCES 47  
  FIGURES AND TABLES 48  
  SUPPLEMENTARY MATERIAL 61  

CHAPTER 4. EFFECT OF INTERLEUKIN 1b, INTERLEUKIN 8 AND INTERFERON GAMMA ON PRRS VIRUS PERSISTENCE 68  
  ABSTRACT 69  
  INTRODUCTION 70  
  MATERIALS AND METHODS 72  
  RESULTS 76  
  DISCUSSION 80
ABSTRACT
The integration of computer technology into research is a continually evolving process. There are many different areas of computer technology. The two main areas that will be discussed here are computer software and databases. Both computer software and databases have multiple languages from which to choose when implementing these technologies. In the current project, the languages used for computer software were the programming language Java and the scripting language PHP. The software package used for the database was MySQL. The program written in Java was a Graphical User Interface (G.U.I.) used to visualize files formatted in the CAP3 format. The PHP script was used to create a website. The website was created to be the interface for connecting to and working with the database. MySQL was used to create the database for the Porcine Reproductive and Respiratory Syndrome (PRRS) Host Genomic Consortium. This database was designed to hold data generated by the Big Pig Project as well as data that will be generated by a new project. Because the data from the Big Pig Project are housed in one database, it is relatively easier to create the file used for statistical analysis. Analysis was done on viral and cytokine, interleukin (IL) 8, IL1b, and interferon gamma (IFNγ) levels and persistence of the Porcine Reproductive and Respiratory Syndrome virus (PRRSv). Results from this analysis indicate that the interaction of cytokines together have an effect on persistency of viral infection or vice versa. Further analysis of the data indicated that swine leukocyte antigen (SLA) genes were associated with cytokine (IL8, IL1b, and IFNγ) response in swine to infection with the PRRS virus. These analyses and results represent an example of using
databases and computer software. Computer technology and research will continue to evolve and the integration of both will continue to grow and become a major component of research and allow for new, inventive ways to study and analyze data.
CHAPTER 1. GENERAL INTRODUCTION

INTRODUCTION

There has always been an intrinsic pursuit of knowledge by mankind. Those in pursuit of this knowledge have always used tools available to them to conduct experiments. In 1942, John Vincent Atanasoff and Clifford E. Berry created the first electronic digital computer, the ABC computer, at Iowa State University [1]. With this invention, came a new tool set available to researchers. As the computer grew in power and popularity, the tool set available from computers grew as well. In 1951, J. Presper Eckert and John Mauchly built the first commercial computer known as the UNIVAC.

One thing that helped facilitate the development of computers was the development of an operating system for the computer. In the early 1950s, General Motors Research Laboratories created the first operating system for their computers and from there operating systems continued to develop to include real-time applications and eventually graphical user interfaces [2]. The popularity and usefulness of the computer continued to grow and this resulted in the creation of programming languages. In 1957, John W. Backus developed the FORTRAN language and compiler [1]. With the development of programming languages, came the development of computer software. Computer software has been designed to both analyze and store data. In the mid-1960s, the software market was thriving, but it was the company known as Applied Data Research that took a major step forward and marketed their software to the customer instead of the producers of the computer [1]. This helped to fuel the development of computer software programs. Computer software continued to encompass new methods of analysis as well as data storage. The data storage eventually evolved into relational
databases. Databases are computer programs designed for specifically storing large amounts of data. Another development that helped to push for continued progression in data storage and communication was the development of the modem by AT&T in the 1960s [1]. This allowed for the digital transmission of data and eventually developed into what is now known as the Internet. With this new form of data communication, computer software developed to allow easier and more efficient communication of data. Then in 1976, Steve Wozniak and Steve Jobs created the Apple I microcomputer, while IBM did not release their personal computer (PC) until 1981 [1]. The invention of the personal computer allowed for the use of computers by individuals in the home. Computers, computer software, databases, and data communication all continue to develop.

AIMS AND OBJECTIVES

The objectives of this project were to: 1) design a software program that would allow the user to view the output, which is in the format of a CAP3 file, of the BLAST Extension and Alignment Program (BEAP) in an easy to interpret format and 2) design and implement a database to store and analyze information from experiments for the PRRS Host Genome Consortium (PHGC). Development of these resources will facilitate evaluation of DNA sequence contigs as well as aid in the organization and analysis of information for the PHGC.

THESIS ORGANIZATION

This dissertation is the result of the author’s own work and research in association with others. The first chapter is a basic introduction and a review of the literature that is associated with the topic of the dissertation. Because the topic focuses on work with databases and computer software, the literature review has been split into
two sections. The first section discusses relational databases and their implementations. The second section covers the development and implementation of computer software.

Chapter 2 has been accepted for publication in the journal BMC Research Notes and Chapters 3 and 4 are papers currently in the process of being prepared for submission for publication. Chapter 2 details the creation of a software package that can assemble small portions of a genome and development of a graphical user interface (G.U.I.) that the end user can use to visualize assembled sequences. Chapter 3 details the statistical analysis, in reference to swine leukocyte antigen genes, of data warehoused within a relational database that was developed. Chapter 4 details the statistical analysis, in reference to persistency and cytokine involvement, of data warehoused within the same database discussed in Chapter 3.

In Chapter 2, the main author was J.E. Koltes with contributions made by E.R. Fritz under the direction of J.M. Reecy. In Chapters 3 and 4 the main author was E.R. Fritz under the direction of J.M. Reecy. The contributions for Chapter 2 were composed of work by J.E. Koltes and Perl scripts and software testing performed by Z-L Hu. The G.U.I. portion was written by E.R. Fritz using Java scripts under the direction of Z-L Hu and J.M. Reecy. Work done for Chapters 3 and 4, contributed by E.R. Fritz, includes a major portion of the writing, development of the database and website, and the majority of the statistical analysis. This work was done in association with D. Nettleton, J. Lunney, and S. Ho under the direction of J.M. Reecy.

REVIEW OF RELEVANT LITERATURE

Databases Models
In the last decade, the amount of data that has been produced from experiments has drastically increased. This increase in the amount of data requires that it be stored in databases as opposed to files and spreadsheets and databases also allow more flexibility for querying the data. This is true even for smaller labs and groups. A database is a repository of information or data that uses a database management system (DBMS) to operate. A DBMS is a software package that manages large collections of data and supports a data model that allows the user to define the structure of data, access data, and update data [3]. The data within a database is not stored randomly in a central location, but follows a schema developed by the user. A schema represents all the relationships that the real world entities in a data source have that a user wishes to include in the database[3]. These relationships are usually developed first as a model that is not the final database schema. An example of such a model is the entity-relationship (ER) diagram (Figure 1). After being developed in this form, the next step is to transfer the relationships to an actual database design.

The entity-relationship diagram is a visualization of the entity-relationship (ER) model developed by Peter Chen [4]. The model contains entities and relationships between those entities. An entity is considered something that can be identified distinctly, while a relationship is the association of these entities [4]. An example of this type of situation would be the pedigree-experiment relationship where a pedigree or pig is used in an experiment as seen in Figure 1. The reason the ER model was developed was because it allowed for a “more natural view in which the world consists of entities and the relationships between them” [4]. So the entities and relationships that are
considered for use are only those that are pertinent to the design of the database and what information is to be stored in it.

**Figure 1.** Example of an ER Diagram – Solid lines indicate attributes that are required while dashed lines indicate attributes that are optional.

When designing a database using the ER model, there are four steps that should be considered. They are: (1) identify all the entities and relationships to be used in the database; (2) identify the semantic information for the relationships, such as one-to-one, one-to-many, many-to-one, or many-to-many; (3) identify the attributes for each entity; (4) identify the relations between the entities and relationships and organize these into the relations and then decide what attributes make up the primary keys [4].

Once the ER model has been chosen and diagrammed, the actual database can then be set up based on the ER diagram. When converting the ER diagram to the database model several things need to be considered: (1) each entity becomes its own table in the database and has the appropriate fields based upon the attributes for that
entity; (2) each relationship needs to be evaluated and the appropriate fields added to
each entity participating in that relationship; (3) the primary keys need to be set based
upon the primary keys selected for the ER model/diagram; (4) foreign keys need to be
set based upon the relationships and the appropriate fields designated for those
relationships in the tables [5].

![ER Diagram](image)

**Figure 2.** An example of the database representation of Figure 1.

Once an ER model and database design have been developed, a decision must
be made as to how and with which database software system to implement these
models. There are several database languages/systems. The systems that will be
discussed in this literature review are: (1) MySQL (SQL); (2) Oracle; (3) dBase; (4)
Microsoft Access; and (5) XML.

MySQL is a relational database system that was developed by Michael Widenius
for the Swedish Company TcX. It was not until 1996 that MySQL was released to the
public in the form of binary distributions for Linux and Solaris [6]. While not being open
source, MySQL does have nonrestrictive licensing that allows use of the product for in
house projects for free [6]. MySQL is also a fast, high performance, and a simple DBMS. This, along with the fact that MySQL supports the SQL query language, supports a variety of programming interfaces, has full networking (i.e. the ability to be accessed from anywhere on the internet), and is portable, have caused MySQL to be a popular and widespread database system [6].

Oracle is database system that started out as relational and is now object-relational. It was founded by Larry Ellison in 1977 as Relational Software Inc [7]. Oracle is supported by Java and supports multiple users and multiple transactions. Oracle uses a System Global Area (SGA) to manipulate its data. An SGA is shared memory and memory structures that all users of an instance of a database use [8]. This database system is in use by most Fortune 500 businesses and quite a few internet businesses [7].

dBase is another database system that was created by the parent company Ashton-Tate. dbase enjoyed widespread use in that it was the first DBMS to be used widely for microcomputers [9]. Currently, dBase is an object oriented language. It can be used on 32 bit Windows and can be used to build applications. dBase databases can be accessed through the use of Open Database Connectivity (ODBC) drivers [9]. The Microsoft ODBC is written in the C programming language and allows applications to connect to many DBMSs, especially relational systems [10].

Microsoft Access is a database system that has a relational base and has been built into the Microsoft Office software package. It has a graphical user interface and tools for software development. Microsoft Access can use information stored in its own database system or in any database that has ODBC compatibility [10].
Extensible markup language (XML) is not a traditional database system. While not an actual markup language, XML has a uniform document structure and is referred to as a metalanguage. This allows it to have more flexibility than traditional markup languages such as html [11]. While XML is not a database in and of itself, with newly developed system/software that can use the innate structure of XML to query and store information, XML forms part of a viable new database system. This system is not a relational system, but makes use of the structure of XML to duplicate the structure of tables in relational databases [5].

While each DBMS may have different applications and functions attributed with them, all database systems have one thing in common, they store data in a more streamlined, easier to access form than flat files. Most of the DBMSs have “constraints” that allows for internal consistency. They also provide a system that allows for multiple users to insert and update information concurrently without any interference in keeping data accurate and up to date. Each DBMS contains a way to handle users and user control. In addition to the previously mentioned options, most DBMSs will have backup and recovery options built in [11].

A relational database scheme consists of a finite amount of relation schemes and a relational database contains a relation for each of these relational schemes [5]. The first part of the relation is the name. This gives the relation an identity and allows for referencing of the relation. Another part of the relation is called an attribute. An attribute is an element of data that exists about a relation. There are usually multiple attributes for a single relation. These attributes are also given a name for identity and referencing.
Another part of the relation is the tuple. A tuple is a specific value set for the attributes associated with a relation. Each tuple has a single value for each attribute.

A very important part of a relation that combines the concept of attributes and tuples is the primary key. The primary key is the set of attributes that are used to uniquely identify each tuple. The primary key is designed based on the data so that it is impossible for any tuple to have the same primary key as another tuple.

Another type of key is the foreign key. Unlike the primary key, there can be multiple foreign keys associated with a single relation. A foreign key is an attribute or set of attributes that is the primary key of another relation within the database.

<table>
<thead>
<tr>
<th>PEDIGREE</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UPN</strong></td>
<td><strong>FarmNumber</strong></td>
<td><strong>Sire</strong></td>
<td><strong>Dam</strong></td>
<td><strong>BirthDate</strong></td>
<td><strong>HarvestDate</strong></td>
<td><strong>Gender</strong></td>
<td><strong>Experiment_ID</strong></td>
</tr>
<tr>
<td>101</td>
<td>354</td>
<td>231</td>
<td>214</td>
<td>05/25/05</td>
<td>05/15/06</td>
<td>M</td>
<td>314</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment_ID</strong></td>
<td><strong>ExperimentName</strong></td>
<td><strong>Virus</strong></td>
<td><strong>Protocol</strong></td>
</tr>
<tr>
<td>314</td>
<td>Big Pig Study</td>
<td>415</td>
<td>12</td>
</tr>
</tbody>
</table>

**Figure 3.** Represents 2 relations with 1 tuple each. Attribute names in bold are primary keys and attributes in italics are foreign keys.

The previously mentioned portions of the database can be referred to by different terms. A relation is often called a table and the attributes and tuples are called columns and rows, respectively. These terms are used interchangeably, but it is important to understand that they are not always used to represent the same things. Tables, columns, and rows are physical representations of relations, attributes, and tuples. While the database itself (relations, attributes, and tuples) does not imply an order or value to the data, the representation of the database (tables, rows, and columns) often do because this order and value is important to the user[12].
With an understanding of the system and complexities of a database, it is easily recognizable that databases provide for the ease of data retrieval. It is this concept of data coming in a single direction when querying a database that allows the ease of connectivity to programs written in a variety of programming languages. The most common relational DBMSs are, directly and indirectly, supported Java, C, C++, Perl and Python [11].

It is this ability to be accessed by a variety of programming languages and the ability to support network access over office networks or the internet that allows for more efficient communication and processing of data when using a relational DBMS.

**Other Computer Software**

It has been previously stated that computer software has been an integral part of database development, access, and sustainability, but computer software can also be designed to be stand alone and has a variety of alternate functions other than simply interfacing with a database.

<table>
<thead>
<tr>
<th>Program Name</th>
<th>Program Language</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>InfluSim</td>
<td>Java</td>
<td>[19]</td>
</tr>
<tr>
<td>THR Simulator</td>
<td>C++</td>
<td>[20]</td>
</tr>
<tr>
<td>BLogo</td>
<td>Perl</td>
<td>[21]</td>
</tr>
<tr>
<td><a href="http://www.nematode.net/FTP/index.php">http://www.nematode.net/FTP/index.php</a></td>
<td>PHP</td>
<td>[22]</td>
</tr>
</tbody>
</table>

**Table 1.** A list of examples of software languages and programs written in those languages.

Table 1 shows that there are a variety of software types and jobs that computer software can accomplish. Computer software can use complex algorithms to analyze, manipulate, and calculate information. An algorithm is a method used to solve a
problem with actions that it executes and an order in which to execute these actions [13]. With the use of these complex algorithms, you can have the computer software do almost anything you want with information. Obviously there are some restrictions, most of these occurring from the syntax used in the specific programming language being used in implementation.

Some computer software also has the ability to connect to databases. This is usually done in terms of an intermediary language that is used to convert the information pulled from the database into a format that the computer software can use. An example of this is Java. A program programmed in Java can connect to SQL and MySQL databases via Java Database Connectivity (JDBC) [5].

Computer software is not only able to connect to databases, but it also has the capability of being able to connect to other software. This allows for the interconnectivity of computers that helps to create networks. A network is defined as collections of computers and transmission channels that allow communications over distances [14]. With networks, data are able to be shared easily and across greater distances than was possible before networks. These networks also allow the ability to connect to a database without the software being contained on the same machine as the database.

There are many computer programming languages available for use and each handles the previously mentioned software abilities in both unique and similar ways. The specific programming languages that will be discussed include: Java, C++, Perl, Basic and PHP.

Java is an object-oriented programming language conceived by Sun Microsystems. An object-oriented programming language is based upon objects that are
created in a program and these objects have methods, which are the operations and functions of the program [13]. Java was developed based upon the C and C++ programming languages, but it was designed to be platform independent unlike C and C++ [15]. To do this, Java compiler output was designed to be in bytecode, which is not recognizable by the computer as an executable language. However, bytecode is a highly optimized set of instructions designed to be executed by the Java run-time system known as Java Virtual Machine (JVM) [15]. This means that Java code written on a computer using one type of operating system will run on a computer with any other operating system, as long as that computer has the JVM on it. It is this concept that gives Java its platform independence. Java was also designed to be able to create interactive, networked programs as well as still allowing for the ability to do high performance operations but it is slower than compiled languages like C++[15]. The introduction of the World Wide Web helped to boost the popularity of Java because of its ease of implementation into web page design. Java is in its sixth implementation and continues with the motto “Write once, run anywhere, any time, forever” [15].

Like Java, C++ can be an object-oriented programming language which is evolved from the C programming language. C++ is a hybrid language though, so it can be used to program in a C-like style, procedural programming. Procedural programming is based on actions that are formed into functions which are, in turn grouped together to form programs [13]. Unlike Java, C++ is platform dependent, which restricts what type of machines can be used to program and run a program written in C++. C++ also has more computing power than Java, meaning that it is able to handle tasks that are more complex and require more memory and system usage [16].
Unlike Java and C++, Perl is not an object-oriented programming language. Originally known as Pearl, it was developed in response to a management requirement for configuration management. Perl is an interpreted language that has been optimized for scanning text files, extracting information from the files and then reporting on that information [17]. Perl was built for practicality instead of the “beauty” that other programming languages offer. It is easy to use, efficient, and minimal but still contains pattern matching, file handling, and formats [17].

BASIC or Beginner’s All-Purpose Symbolic Instruction Code was developed as a way of writing simple programs so that it could be used to teach programming techniques. Microsoft Visual Basic was developed based on this idea and was created to make Windows applications [13]. Visual Basic also happens to be one of the most popular programming languages in the world.

PHP, unlike the aforementioned programming languages is a scripting language designed only for use in creating Web pages. PHP, also known as PHP:HyperText Preprocessor, uses a syntax of that similar to the C programming language, but is a lot simpler than the C language [18]. PHP is growing in popularity due to its ability to interact with databases.
REFERENCES


   BMC Infect Dis. 2007. 7: p. 17.


CHAPTER 2. BEAP: THE BLAST EXTENSION AND ALIGNMENT PROGRAM- A TOOL FOR CONTIG CONSTRUCTION AND ANALYSIS OF PRELIMINARY GENOME SEQUENCE

A paper published to BMC Research Notes

James E. Koltes¹, Zhi-Liang Hu¹, Eric Fritz¹, and James M. Reecy¹

¹Department of Animal Science, Iowa State University, Ames, Iowa 50011-3150, USA

ABSTRACT

Background

Fine-mapping projects require a high density of SNP markers and positional candidate gene sequences. In species with incomplete genomic sequence, the DNA sequences needed to generate markers for fine-mapping within a linkage analysis confidence interval may be available but may not have been assembled. To manually piece these sequences together is laborious and costly. Moreover, annotation and assembly of short, incomplete DNA sequences is time consuming and not always straightforward.

Findings

We have created a tool called BEAP that combines BLAST and CAP3 to retrieve sequences and construct contigs for localized genomic regions in species with unfinished sequence drafts. The rational is that a completed genome can be used as a
template to query target genomic sequence for closing the gaps or extending contig sequence length in species whose genome is incomplete on the basis that good homology exists. Each user must define what template sequence is appropriate based on comparative mapping data such as radiation hybrid (RH) maps or other evidence linking the gene sequence of the template species to the target species.

Conclusions

The BEAP software creates contigs suitable for discovery of orthologous genes for positional cloning. The resulting sequence alignments can be viewed graphically with a Java graphical user interface (GUI), allowing users to evaluate contig sequence quality and predict SNPs. We demonstrate the successful use of BEAP to generate genomic template sequence for positional cloning of the Angus dwarfism mutation. The software is available for free online for use on UNIX systems at http://www.animalgenome.org/bioinfo/tools/beap/.

FINDINGS

METHODS

BEAP Construction

The BLAST and CAP3 processes were linked via Perl scripts to create a sequence assembly pipeline (see Additional file 1 for rationale and Additional File 1 Figure 5.) BLAST was looped in an iterative process such that after all the queries, each unique sequence was used as template for the next round of BLAST against the initial databases. After all sequences were retrieved, they were sent to CAP3 for assembly using the default settings and available sequence quality files from NCBI. The CAP3 output, saved as a text file, can then be manually uploaded using the BEAP GUI. A
detailed discussion of results from testing of BEAP options is presented in Additional File 1.

Several internal features were created to enhance and monitor BEAP performance. A filter was created to remove sequences retrieved multiple times to limit the number of frivolously repeated BLAST queries. The number of BLAST rounds was also monitored such that only the desired number of reiterative BLAST rounds was performed, up to a maximum number defined by the user. This method allowed BEAP to stop the re-iterative BLAST process if no new sequences were retrieved, reducing the amount of time needed to complete a BEAP assembly. A progress summary was also kept in the log file throughout the process to keep track of sequences retrieved and corresponding statistics such as sequence ID, database or origin, number of bases, E-value, etc.

Development of the BEAP GUI for sequence alignment visualization

The BEAP GUI was built as extension of BEAP to improve assembled sequence analysis. The GUI allows a more in depth examination of individual sequences and contigs in more viewer friendly environment for those not used to raw program output. The GUI consists of a main window that is opened upon execution of the program. Features include a button for contig file uploads, a selection box to change contig views and a viewer panel in which the contig and all the sequences used to create it are visualized.

Visualization of contigs and their accompanying sequence members was accomplished by parsing through the output files generated by CAP3 using the designations for sequence IDs from NCBI, ie. CO, BQ, AF, RD, and QA. The program
uses the parsed information from the CAP3 file to create a contig object that has associated an array of sequence objects composed of the sequence that was used to create this contig. The contig object itself is then put into an array. The program then passes the array of contig objects to the other parts of the program responsible for creating views, saving images of these views, and printing of the views.

**Software Development**

BEAP is written in Perl 5.8.0 and tested on a Red Hat Linux Application Server 3.0. The NCBI BLAST (version 2.2.9) and CAP3 (version date: 08/29/2002) software are utilized by BEAP to serve the purpose of sequence searching and assembling. Currently, BEAP is a command line program run on a Linux terminal. Planning is under way that it will be implemented as a web interfaced program for public access. The GUI tool was developed with Java (version 1.5.X) and tested on Windows XP and Mac OS X.4.11. The BEAP program was developed and tested on a 533 MHz dual processor Linux computer with 8 GB RAM.

**BEAP Performance Testing**

Since many options were inherently flexible in BEAP, we wanted to test a number of scenarios that could alter BEAP performance. To determine how BEAP output was changed when BLAST settings were altered, we varied the E-value stringency, word size and number of databases. To determine how different sequence attributes would affect BEAP performance, we compared output generated when using intronic, exonic, exonic and untranslated regions (UTR), and varying levels of repetitive sequence elements template sequence. To determine how template sequence size altered BEAP performance, we tested individual template sequence sizes, the number
of sequences used as template, and the total amount of sequence used as template. Last, we investigated the difference in BEAP output when using local (megaBLAST) vs. network BLAST query.

**Template Sequence**

The user must define the appropriate template sequence and species. The template sequence is used much like primers in PCR for BLAST to query the species of interest. Cross-species comparative maps (i.e. RH maps) can be used to identify syntenic sequence blocks between species to find a suitable template sequence.

**Application: The Use of BEAP to Construct Contigs Within the Angus Dwarfism Locus**

Fine-mapping of the Angus dwarfism locus resulted in a critical region of roughly 1-2 Mbps on *Bos taurus* autosome (BTA) 6. Since the bovine genome was not fully sequenced upon the first application of BEAP in 2005, many of the candidate genes in this genomic region were unknown and unannotated. The Human-Bovine RH map was used to define the template sequence allowing for some extra sequence proximal and distal to the homologous bovine chromosome block. *Homo sapien* autosome (HSA) 4 genomic DNA sequence from 78,000,000 to 83,000,000 base pairs was defined as the “template” for BEAP assembly of the bovine. This genomic block contained 20 genes and pseudo-genes [1, 2]. The template sequence used by BEAP included both exonic and UTR sequences. All template sequences were retrieved from UCSC genome browser [3] and the ENSEMBL database [4]. Repetitive sequences were masked using Repeat Masker software in the template (i.e. human sequence) prior to use of BEAP [5]. We used RH markers within genes in both the bovine and human genome builds to
anchor the template to the target physical map. An E-value of e-30 was used for all tests. We queried sequence from six NCBI databases (see Additional File 1). The sequences obtained in the application to the bovine dwarfism locus used the sequence databases available in 2005, prior to full bovine genome assembly. The BEAP performance trials utilized the whole bovine genome sequence, version 3, from 2007.

RESULTS

Performance of BEAP

BEAP test results indicated contigs spanning exons and flanking intronic sequences, allowing researchers to find gene sequences in contigs. However, in some cases, it may be necessary to check BEAP singlets for exonic sequence if little sequence homology or no flanking sequence exists in the introns of genes. Template sequence comprised of up to 1-5 Mbs total bases can be used to construct contigs depending on computing power and E-value stringency level desired. Changing the E-value and word size generally resulted in the expected result- more sequences queried with a lower E-value or word size and less sequence queried with a higher E-value or larger word size (see Additional File 1.) Clearly, it is important to consider the specificity and precision of the retrieved gene sequence in choosing a lower or higher E-value in BEAP respectively. Both local and network (more current, web-based) databases can be used to query sequence for assembly with little difference in performance. Repetitive template sequence had a limited effect on BEAP, with some reduction in contig size and number as percentage of repetitive sequence content increased.

Performance of the BEAP GUI
The BEAP GUI allows for easy identification of sequence mismatch that may represent possible SNPs. In the nucleotide view, an option is present to colour base mismatches between sequence reads. This nucleotide mismatch tool can be turned on or off as desired by the user. A base in the sequence data that does not match the equivalent base of the contig is coloured either red or green when this feature is turned on. A red base means that the base does not match, but the score for that base in the contig is high (>50). A green base means that the base does not match, and the score for that base in the contig is low (<50). Identification of sequence variation may indicate the presence of SNPs, poor sequence quality, or an improperly placed sequence within a contig. The ability to code these sequences allows a researcher to assess the quality of the sequence assembly and the potential of polymorphic nucleotide variation. The ability to detect SNPs would have immediate impact as genetic markers to expedite alignment of contigs to genetic maps or for identification of gene duplication. Refinement of this tool is required to add confidence in a single mismatch as a putative SNP.

**Application: The Assessment of the Bovine Dwarfism Locus Assembly**

Table 1 presents a summary of the genes retrieved, their sizes and a sequence similarity comparison to human. The sequence similarity between human and cattle ranged from 85.3% to 95.1%. An example of assembled sequence is presented in Figure 1 along with an alignment of an individual sequence member to a constructed contig using NCBI’s BLAST 2 sequences tool [6] to demonstrate how BEAP extended template sequences. Analysis of the Angus cattle dwarfism locus demonstrates the potential application of BEAP in a low draft sequence. Our initial test using pre-genome
assembly sequence from cattle created a large number of contigs, encompassing the vast majority of genic regions and flanking intronic sequence for the dwarfism locus. Nineteen genes were identified in cattle based on BLAST results (Figure 1). Use of current sequence draft data for the cow also created a wealth of sequence data for positional cloning of the locus. The ability to use local and network databases shows the flexibility of BEAP for different researchers' needs. The sequence data created was used to design primers for positional candidate gene analysis and eventually for the discovery of the causative mutation for Angus dwarfism (Koltes et al., submitted manuscript).

DISCUSSION

BEAP: A Foundation for Comparative Based Genomic Characterization

A web-based interface is currently being planned to allow wider public access to BEAP. Wider access to BEAP will greatly expedite analysis of draft genomes, which would otherwise require considerable individual effort. To facilitate retrieval of template sequence, we plan to provide links to ENSEMBL, TIGR, NCBI, UCSC, and RH maps relevant to livestock species available at the NAGRP website [7]. Livestock sequence databases are also available through these links. Links to plant databases, including TAIR [8] and plant genome db [9] could also be provided. Additionally, we would like to add a front end template sequence processing component to screen template sequence for repetitive sequence elements facilitated by repeat masker [5]. If desired, users could choose other contig and sequence assembly viewers, e.g [10, 11, 12].

Downstream applications of BEAP output would include links to query NCBI, UCSC and ENSEMBL to view map positions and exon/ intron boundaries of genes. Alternatively, access to these databases is facilitated by ECR viewer [13], part of the
dCode project, which finds evolutionarily conserved sequences and provides graphical sequence analysis using Zpicture, rVista, and Mulan [14]. In addition, if high homology is present between template and query sequence, programs such as Mulan can use comparative genomics to assess the localized quality of sequence assembly using mega dot plots comparing template and queried sequences for limited distances. This software also allows cross-species annotation. The Avid software could serve to order BEAP contigs [15].

During the development of BEAP, a similar tool was published to harness trace sequences for assembly into contigs [16]. BEAP differs from this software in that it works locally, and therefore more quickly. It also allows users more flexibility in BLAST options including a wider variety of searchable sequence databases. BEAP also facilitates visualization and quality assessment of sequences and detection of possible SNPs.

CONCLUSIONS

Researchers need to have some flexibility to adapt to each new genome depending on genomic complexity and gene content. A variety of options in the BEAP process were designed to help researchers tackle a wide range of challenges. BEAP is not limited to bovine or animal applications. Any sequence database can be queried or used as a template sequence. The user can specify local (megaBLAST) or remote (BLASTn) database querying, stringency of BLAST hits (E-value), and word size within the megaBLAST option. Users have great flexibility in how to use BEAP output. The BEAP package could be very useful to researchers working with draft quality genome sequence.
COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR’S CONTRIBUTIONS

Z-LH designed, updated and participated in testing of the software and provided the vision to create the viewer. JEk conceived ideas to build and enhance the software and performed software tests and drafted the manuscript. EF created the BEAP GUI and assisted with writing the manuscript. JMR conceived the idea of the software, assisted in conceptual improvement of the software, as well writing of the manuscript.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Xiaoqiu Huang, Dr. Scott Emrich, and Dr. LaRon Hughes of Iowa State University and Dr. Denis Bauraing of University of Liege, Liege, Belgium for their discussions and input into the creation and improvement of BEAP.

REFERENCES


3. *The UCSC Genome Browser.* [http://genome.ucsc.edu/]


    [http://www.animalgenome.org/bioinfo/resources/manuals/contigimage.html]

12. *Gap 4 Contig Viewer.*


FIGURES

**Figure 1 – Demonstration of sequence extension using BEAP**

Example of a contig created in the Angus dwarfism locus by BEAP. The contig is aligned, using the align two sequences tool at NCBI [16], to the first BLAST match to the template sequence retrieved from NCBI. The alignment shows both successful retrieval of target sequence in cattle and nearly tripling in the size of the template for primer design and lab analysis (883 bps for individual sequence vs. 2237 bps for constructed contig). The score, expect, identity and gap statistics show the contig was used in its entirety, with no gaps and 100% sequence match.

TABLES

**Table 1 - Bovine genes discovered in the Angus dwarfism locus by BEAP (prior to bovine assembly.)**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Size*</th>
<th>% Similarity to Human*</th>
<th>% of transcript retrieved*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANTXR2</td>
<td>167.52 (3551)</td>
<td>90.6</td>
<td>29.7</td>
</tr>
<tr>
<td>ANXA3</td>
<td>58.69 (1443)</td>
<td>93.3</td>
<td>22.9</td>
</tr>
<tr>
<td>BMP2K</td>
<td>102.55 (2158)</td>
<td>91.0</td>
<td>35.5</td>
</tr>
<tr>
<td>BMP3</td>
<td>22.61 (1774)</td>
<td>89.4</td>
<td>47.9</td>
</tr>
<tr>
<td>COPS4</td>
<td>40.73 (1747)</td>
<td>93.7</td>
<td>100</td>
</tr>
<tr>
<td>FGF5</td>
<td>24.33</td>
<td>89.8</td>
<td>100</td>
</tr>
<tr>
<td>Gene</td>
<td>Genomic Size (kbs)</td>
<td>Transcript Size (bps)</td>
<td>Sequence Similarity</td>
</tr>
<tr>
<td>--------</td>
<td>--------------------</td>
<td>-----------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>FRAS1</td>
<td>486.29 (15213)</td>
<td>90.8 21.2</td>
<td></td>
</tr>
<tr>
<td>GDEP</td>
<td>34.2 (521)</td>
<td>86.0 100</td>
<td></td>
</tr>
<tr>
<td>GK2</td>
<td>1.67 (1677)</td>
<td>87.9 100</td>
<td></td>
</tr>
<tr>
<td>HEL308</td>
<td>48.50 (3559)</td>
<td>92.9 59.1</td>
<td></td>
</tr>
<tr>
<td>HNRPD</td>
<td>20.62 (2200)</td>
<td>95.1 100</td>
<td></td>
</tr>
<tr>
<td>HPSE</td>
<td>42.69 (3705)</td>
<td>93.3 66.4</td>
<td></td>
</tr>
<tr>
<td>MASA</td>
<td>24.63 (5113)</td>
<td>89.7 9.2</td>
<td></td>
</tr>
<tr>
<td>MRPL1</td>
<td>89.95 (1165)</td>
<td>89.9 24.6</td>
<td></td>
</tr>
<tr>
<td>PAQR3</td>
<td>21.48 (3990)</td>
<td>92.8 90.7</td>
<td></td>
</tr>
<tr>
<td>PLAC8</td>
<td>24.7 (1391)</td>
<td>- 0</td>
<td></td>
</tr>
<tr>
<td>PRDM8</td>
<td>24.04 (2536)</td>
<td>91.6 76.5</td>
<td></td>
</tr>
<tr>
<td>PRKG2</td>
<td>116.38 (3328)</td>
<td>94.6 100</td>
<td></td>
</tr>
<tr>
<td>SCD4</td>
<td>138.88 (1994)</td>
<td>90.1 100</td>
<td></td>
</tr>
<tr>
<td>THAP9</td>
<td>19.53 (3627)</td>
<td>90.0 13.8</td>
<td></td>
</tr>
</tbody>
</table>

1. The genomic sequence, introns and exons, is given in kilobases (kbs) and the transcript size is presented in parenthesis, in bps. All data was queried in January of 2005, prior to completion of the bovine genome. The size estimates are based on human orthologs as found at UCSC genome browser at http://genome.ucsc.edu/

2. The sequence similarity between human and bovine genes was determined by comparing exonic sequence only.

3. The percentage of transcript retrieved by BEAP is based on the size of the human transcript.
Chapter 3: The effect of swine leukocyte antigen (SLA) genes on Porcine Reproductive and Respiratory Syndrome virus (PRRSV) immunity: viral, serum neutralizing antibody, and cytokine levels

Eric R Fritz¹#, Joan K. Lunney²*, Dan Nettleton³, James M Reecy¹, Chak-Sum Ho⁴, Douglas M Smith⁴, Michal Wysocki²,⁵, Daniel Kuhar², Elizabeth Prucnal², Ramon Molina⁶, Jane Christopher-Hennings⁷, Jeffrey Zimmerman⁶, Raymond R R Rowland⁸

¹Department of Animal Science, Iowa State University, Ames, IA 50011, USA
²USDA ARS APDL, BARC, USDA, Beltsville, MD
³Department of Statistics, Iowa State University, Ames, IA 50011-3150, USA
⁴Pathology Department, University of Michigan Medical School, Ann Arbor MI
⁵Current address:
⁶Iowa State University, Ames IA
⁷South Dakota State University, Brookings SD
⁸Kansas State University, Manhattan, KS

*Corresponding Author: Joan K. Lunney

# ERF contributions include development and implementation of the database as well as statistical analysis.
Abstract

Infection with Porcine Reproductive and Respiratory Syndrome virus (PRRSV) elicits a response that can be partially attributed to the genetics of the host. We hypothesized that viral, serum neutralizing antibody (SNA), and serum interleukin-8 (IL-8), IL-1b, and interferon gamma (IFNγ) levels were in some way related to swine leukocyte antigen (SLA) genotypes and haplotypes. We tested serum samples collected as part of the “Big Pig” project in order to analyze viral replication and immunity in a population of 109 pigs (and 60 control pigs), which were sampled for up to 203 days post-PRRSV infection (DPI). Viral, SNA, IL-8, IL-1b, and IFNγ levels were calculated over the course of the infection; SLA genotypes and haplotypes were determined for most of the infected pigs included in this study. Statistical analyses were then conducted to determine whether there are associations between viral, SNA, IL-8, IL-1b, and IFNγ levels with SLA genotypes and haplotypes. Several SLA haplotypes could be affirmed statistically as potentially affecting one or more of the attributes, e.g., Class 1 Hp 1.0 with increased viral levels and class II Hp 0.1, 0.23 and 0.26 with decreased SNA. These results set the stage for identifying genetic alleles that affect PRRSv infection and anti-viral responses.

Introduction

Swine leukocyte antigen (SLA) genes are important determinants of immune, infectious disease and vaccine responses (Lunney et al., 2009). Based on studies using SLA defined and SLA inbred lines of pigs it was affirmed that SLA genes determined levels of antibody responses to defined protein and vaccine antigens (Mallard et al.,
1989; Vaiman et al., 1998). Due to the difficulty and expense of performing controlled SLA based disease studies only limited numbers of cancer or pathogen challenge studies have been performed (Madden at al., 1990; Blangero et al., 1996; Geffrotin et al., 2004). Over the last decade the complexity of the SLA complex has been defined, including its complete sequence (Renard et al., 2006). As with most mammalian MHC systems, the SLA system is highly polymorphic; molecular techniques have facilitated assignment of SLA alleles and haplotypes (sets of linked SLA class I or class II alleles) (Smith et al. 2005 a,b; Ho et al., 2009a). As documented on the Immuno Polymorphism Database (IPD)-MHC SLA sequence database (http://www.ebi.ac.uk/ipd/mhc/sla/) there are 116 classical SLA class I (SLA-1, SLA-3 and SLA-2) alleles, which are present in 28 different haplotypes; for class II (DRB1, DRA, DQB1 and DQA) loci 129 alleles have been documented with 21 different haplotypes (Ho et al., 2009a). Recent updates to molecular typing for SLA class I and II genes have enabled scientists to probe details of SLA associations with infectious disease responses (Ho et al., 2009b). These advances have enabled researchers to expand studies to assess the effects of specific SLA alleles on quantitative trait loci (QTL) and disease and vaccine responses and to identify exactly which genes are associated with pig resistance to infection by specific pathogens.

This manuscript addresses SLA influences on pig responses to Porcine Reproductive and Respiratory Syndrome virus (PRRSV) infection. PRRS virus elicits a slow and weak immune response, subverting the innate immune system and resulting in slow viral clearance and persistently infected swine (Murtaugh et al., 2002; Mateu and Diaz, 2008). It is clear that humoral neutralizing antibody responses are necessary for
effective PRRS responses (Lopez and Osorio, 2004; Mulupuri et al., 2008). Full protective immunity requires anti-viral response pathways, particularly those regulating the timing and expression of interferon-gamma (IFNγ) responses after PRRSV infection (Bautista and Molitor, 1997; Xiao et al. 2004; Royaee et al. 2004; Wesley et al. 2006). Attempts to identify pigs with genetic resistance to PRRS have revealed differences among and within breeds in response to infection with PRRSV (Halbur et al. 1998; Petry et al. 2005; 2007; Vincent et al. 2005; 2006; Lewis et al., 2007). Petry et al. (2007) identified serum interleukin-8 (IL-8) levels preinfection as a potential predictor of PRRS resistance.

Molina et al. (2008a,b) documented results from the 165 pig “Big Pig” study of viral clearance and persistence over 202 days of infection. This manuscript presents the diversity of SLA class I and II alleles and haplotypes in these pigs and probes statistical association of SLA complexity with anti-viral responses in 101 of 109 infected commercial pigs.

**Materials and Methods**

*Animal care, handling, and sampling*

As previously described by Molina et al. (2008a), 109 Large White/Landrace cross bred pigs were infected with PRRSV and followed for 202 days. Specifically, on day 0, pigs \( n = 109 \) were intramuscularly inoculated with 1 ml of PRRSV (VR-2332 1 X \( 10^{4.0} \) TCID\(_{50}\)) diluted in minimum essential medium. Control pigs were inoculated with PRRSV-negative MEM by the same method. Blood samples were collected from the pigs on day 0 before inoculation, at 7 and 14 days post-inoculation (DPI), and every 14
days after up to DPI 202. For this study DNA was prepared for SLA typing and results used for statistical analyses \((n = 101)\); samples from only 44 pigs were used in the analysis of cytokines \((n = 44)\).

**Reverse-transcriptase polymerase chain reaction**

As previously described by Molina et al. (2008b), quantitative RT-PCR was used to determine viral levels from serum; quantification of the samples was calculated and expressed as number of RNA copies per ml for fluid samples or number of RNA copies per gram for solid tissue samples.

**Serum Neutralizing Antibody (SNA)**

Through the use of a modified fluorescent focus neutralizing (FFN) assay, PRRSV-neutralizing activity in serum and muscle samples was measured as previously described by Molina et al. (2008a). Two-fold dilutions \((1:4 \rightarrow 1:512)\) of heat-inactivated pig serum were tested with homologous virus \((ATCC VR-2332)\). The neutralizing titer level was recorded as the last dilution that showed 90% or greater reduction in the number for fluorescent foci.

**Cytokine protein analysis**

Interleukin (IL) 1b, IL-8, and interferon gamma (IFN\(\gamma\)) were quantified in sequential serum samples collected at 14-day intervals from 44 PRRSV-inoculated animals. Interleukin (IL) 1b, IL-8 were evaluated in samples collected through DPI 42 and IFN\(\gamma\) in samples collected through DPI 84. Serum samples were assayed with
commercial ELISA kits (IL-1b and IL-8: R&D Systems, Minneapolis, MN, USA 2; IFNγ: Biosource™ Invitrogen, Camarillo, CA, USA) following protocols provided by the manufacturers. For all ELISA data, the OD of the medium controls was averaged and subtracted from the OD of each sample and then the lowest readable standard concentration was determined from the standard curve. If the OD was below the lowest part of the curve, values were assigned by dividing the lowest readable standard concentration by 2 and then multiplying that number by the dilution factor (i.e. 2, 4, or 10).

**Swine Leukocyte Antigen (SLA) characterization**

Total DNA for swine leukocyte antigen (SLA) characterization was isolated from lymphoid tissues (superficial inguinal and submandibular lymph nodes) using the Qiagen® DNeasy Blood and Tissue kit spin-column protocol following the instructions provided by the manufacturer. Swine leukocyte antigen (SLA) class I and class II genotypes were determined in 101 and 93, respectively, of the 109 PRRSV-inoculated pigs using a “one-step” PCR approach (Ho et al., 2009a; Supplementary Materials). This method relies on a set of sequence-specific PCR primers designed to differentiate SLA alleles by groups with similar sequence motifs (i.e., low-resolution SLA typing) as assigned by the SLA Nomenclature Committee of International Society for Animal Genetics (ISAG) for the DNA sequenced alleles (Smith et al., 2005 a,b; Ho et al., 2009 a,b). PCR reactions, thermal cycling parameters and gel electrophoresis were performed as previously described (Ho et al. 2009a; Martens et al. 2005). The DNA-sequenced SLA haplotypes and the SLA haplotypes previously identified in nearly 700
pedigreed pigs obtained from multiple commercial sources using the same typing method were also taken into consideration for resolving ambiguities (Smith et al., 2005 a,b; Ho et al., 2009 a,b).

Statistical analysis

For each animal, we computed the area under the curve of the log of virus, SNA, IL-8, IL-1b, and IFNγ levels from 0 to 42 dpi and analyzed each of these as separate response variables. We then computed the log of viral, SNA, IL-8, IL-1b, and IFNγ levels for each day and analyzed each of these as separate response variables. For each response variable, we fit a linear model using R (www.r-project.org).

\[ y = Xa + e \]

where \( y \) denotes the response vector with one element for each animal; \( X \) denotes the design matrix with one row for each animal and one column for each allele; \( a \) denotes a vector of allele effects \((a_1, ..., a_{16})\) with one element for each allele; and \( e \) denotes a vector of independently and normally distributed mean-zero error terms with a constant unknown variance. The entries in a row of \( X \) give the number of copies of each allele carried by an animal. For example, the row corresponding to an animal that is homozygous for the first allele will have the entries 2, 0, ..., 0; while the row for an animal that is heterozygous with the first and second alleles will be 1, 1, 0, ..., 0. Thus, our model indicates that \( 2a_1 \) is the mean response of animals carrying two copies of allele 1, and \( a_1 + a_2 \) is the mean response of heterozygous animals carrying alleles 1 and 2.
For each response variable, standard linear model $F$-tests were constructed for testing the equality of all allele effects, the difference between each allele effect and the average of the others, and all pair-wise comparisons between allele effects. The $p$-values from the latter two sets of tests were corrected to control the family-wise error rate using the Bonferroni method, and corrected to control the false discovery rate (FDR) using Benjamini and Hochberg’s (1995) method, although only the FDR corrected $p$-values are reported.

Database information

The PRRS Host Genome Consortium (PHGC) database was developed to warehouse all data collected in the “Big Pig” experiment (Molina et al., 2008 a,b) and to serve as the future repository of PHGC data. The database was created using MySQL and is hosted on a Linux server. The structure of the database was designed based upon the data generated by the Big Pig Project and allows for the inclusion of new data, e.g., SNP chip and microarray data that are expected to be generated in future PHGC studies (Figure 1).

Associated with the database is a web-page interface. The web-page was written with PHP and JavaScript, is hosted on the same server as the database, and can be accessed via the internet (http://www.animalgenome.org/lunney/index.php). The web-page interface allows access to the database anywhere in the world, while maintaining control over database management and security. This website also allows approved users to enter and retrieve data. This control and security is provided via a user account accessible through use of the web-page interface.
Results

SLA haplotype effects on virus and SNA levels

The SLA class I and class II alleles present in the “Big Pig” population were determined by low resolution PCR techniques. Because of the lack of pedigree information for these pigs, haplotypes were deduced based on the identification of animals homozygous for SLA genotypes followed by the subtraction of these haplotypes in animals that were heterozygous (Table 1, 2). Data on virus levels from 7 to 42 dpi and SNA were summarized based on log of area under the curve and the effect of each SLA class I or II haplotype estimated using the model noted (Tables 3, 4).

The significance of the overall effect of the SLA Class I haplotypes on virus level was $p<0.0001$ (Table 3). The individual haplotypes 4 (Hp4.0), Hp30.0, and Hp32.0 had a significant effect at $p<0.05$ although the number of pigs with sufficient representation is only sufficient for Hp4.0. The overall effect of SLA Class I haplotypes on SNA level was significant, $p<0.05$; however, no individual haplotype was significantly different than zero (Table 3).

The overall effect of SLA Class II haplotypes on virus level was significant, $p<0.01$, with individual haplotype Hp0.12 having a significant effect at $p<0.05$ (Table 4). The overall effect of SLA Class II haplotypes on SNA level was significant, $p<0.05$, with individual haplotypes Hp0.1, Hp0.23, and Hp0.26 all having suggestive effects, $p<0.10$ (Table 4).

SLA Haplotype effects on IL-8, IL-1b, and IFNγ levels
Area under the curve was calculated for IL-8, IL-1b, and IFNγ levels and the effect of each SLA class I or II haplotype estimated for IL-8, IL-1b, and IFNγ (Tables 5, 6). The overall effect of SLA Class I haplotypes on IL-8 levels was only suggestive, $p<0.10$, with individual Hp30.0 having a significant effect, $p<0.05$ (Table 4). The overall effect of SLA Class I haplotypes on IL-1b level was significant, $p<0.01$, with individual Hp1.0 having a suggestive effect, $p<0.10$, and Hp31.0 having a significant effect, $p<0.05$ (Table 5). The overall effect of SLA Class I haplotypes on IFNγ level was not significant, consequently no individual haplotype had a significant effect (Table 5).

The overall effect of SLA Class II haplotypes on IL-8 level was not significant, and no individual haplotype had a significant effect (Table 6). The overall effect of SLA Class II haplotypes on IL-1b level was significant, $p<0.0001$, with individual haplotypes Hp0.23, Hp0.25, and Hp0.26 having significant effects, $p<0.05$ (Table 6). The overall effect of SLA Class II haplotypes on IFNγ was significant, $p<0.05$, with individual haplotypes Hp0.4, Hp0.13, and Hp0.25 all having suggestive effects, $p<0.10$ (Table 6).

SLA genotype effect on virus and SNA levels

The overall effect of the alleles of Class I SLA-1 was significant for virus, $p<0.05$. However, there were no significant individual allele effects on virus or SNA levels (Supplementary Materials).

The overall effect of the alleles of Class I SLA-2 was significant for SNA, $p<0.05$ (Table 4), but not virus, $p>0.05$. There were no significant individual allele effects on virus levels (Supplementary Materials). Individual SLA-2 alleles w09XX, 10XX, and 12XX, all had significant effects, $p<0.05$, on SNA level.
The overall effect of the alleles of Class I SLA-3 was not significant for virus or SNA. There were no significant individual allele effects on virus or SNA levels (data not shown). The overall effect of the alleles of Class II DRB1 was significant for virus level, p<0.001, with the individual alleles 04XX, 06XX, and 11XX having a significant effect, p<0.05, and allele 07XX having a suggestive effect, p<0.10 (Table 8). The overall effect of the alleles of Class II DRB1 was significant for SNA level, p<0.01, with the individual alleles 01XX, 04XX, 10XX, and 11XX having a significant effects, p<0.05, and allele 07XX having a suggestive effect, p<0.10 (Table 8).

The overall effect of the alleles of Class II DQB1 was significant for virus level, p<0.01, with the individual alleles 03XX and 07XX having significant effects, p<0.05, and individual alleles 02XX and 04XX having suggestive effects, p<0.10 (Table 10). The overall effect of the alleles of Class II DQB1 was not significant for SNA level, but individual allele 06XX did have a significant effect, p<0.05 (Table 10).

The overall effect of the alleles of Class II DQA was significant for virus level, p<0.05. However, there were no significant individual allele effects on virus or SNA levels (data not shown).

Genotype effects on IL-8, IL-1b, and IFNγ levels

The overall effect of the alleles of Class I SLA-1 was significant for IL-8 and IL-1b, p<0.05. However, there were no significant individual allele effects on IL-8, IL-1b, or IFNγ levels (Supplementary Materials).
The overall effect of the alleles of Class I SLA-2 was not significant for IL-8, IL-1b, or IFNγ. There were no significant individual allele effects on IL-8, IL-1b, or IFNγ levels (Supplementary Materials).

The overall effect of the alleles of Class I SLA-3 was significant for IL-1b, p<0.05. However, there were no significant individual allele effects on IL-8, IL-1b or IFNγ levels (data not shown).

The overall effect of the alleles of Class II DRB1 was not significant for IL-8 level and no individual allele effects were significant (Table 9). The overall effect of the alleles of Class II DRB1 was significant for IL-1b level, p<0.0001, with the individual alleles 04XX, 10XX, 11XX, and 13XX having significant effects, p<0.05 (Table 9). The overall effect of the alleles of Class II DRB1 was significant for IFNγ level, p<0.0001, with the individual allele 13XX having a significant effect, p<0.05, and individual allele 02XX having a suggestive effect, p<0.10 (Table 9).

The overall effect of the alleles of Class II DQB1 was not significant for IL-8 level, but individual allele 01XX had a significant effect, p<0.05, and individual allele 03XX had a suggestive effect, p<0.10 (Table 11). The overall effect of the alleles of Class II DQB1 was significant for IL-1b level, p<0.0001, with individual alleles 02XX, 04XX, 06XX, and 09XX having a significant effect, p<0.05 (Table 11). The overall effect of the alleles of Class II DQB1 was significant for IFNγ level, p<0.05, with individual alleles 02XX, 03XX, 04XX, 06XX, and 09XX having significant effects, p<0.05 (Table 11).

The overall effect of the alleles of Class II DQA was significant for IL-1b level, p<0.05. However, there were no significant individual allele effects on IL-8, IL-1b, or IFNγ levels (data not shown).
**Haplotype effect by day on Virus and SNA**

The effect of SLA Class I on virus level was significant for DPI 7 and DPI 14, \(p<0.0001\), but was not significant for DPI 28 or DPI 42 (Table 12). The effect of individual haplotypes 5 and 30 on virus level were significant for DPI 7, \(p<0.05\). The effect of individual haplotype 30 on virus level was significant, \(p<0.05\), while the effect of individual haplotypes 4, 22, 29, and 32 were suggestive, \(p<0.10\), for DPI 14. There were no significant individual haplotypes for DPI 28 or DPI 42 for virus level.

The effect of SLA Class I on SNA level by day was not significant for DPI 28, DPI 42, DPI 56, or DPI 70, but was significant for DPI 64, \(p<0.05\). There were no individual haplotypes for DPI 28, DPI 42, DPI 56, DPI 70, or DPI 84 that were significant (data not shown).

The effect of SLA Class II on virus was significant for DPI 7 and DPI 14, \(p<0.05\), but was not significant for DPI 28 or DPI 42 (Supplementary Materials). The effect of individual haplotype 25 on virus level was significant, \(p<0.05\), for DPI 7 and the effect of individual haplotype 12 was significant, \(p<0.05\) for DPI 14.

The effect of SLA Class II on SNA level by day was not significant for DPI 28, DPI 42, DPI 56, or DPI 70, but was significant for DPI 84, \(p<0.05\). The effect of individual haplotype 0.15b was significant, \(p<0.05\), and the effect of individual haplotypes 0.1 and 0.25 were suggestive, \(p<0.10\), for DPI 84 (Supplementary Materials).

**Haplotype effect by day on IL-8, IL-1b, and IFNγ**
The effect of SLA Class I on IL-8 level by day was not significant for DPI 7, DPI 14, DPI 28, or DPI 42. There were no individual haplotypes for DPI 7, DPI 14, DPI 28, or DPI 42 that were significant (data not shown).

The effect of SLA Class I on IL-1b level was significant for DPI 7, DPI 28, and DPI 42. This effect was not significant for DPI 14 (Supplementary Materials). The effect of individual haplotype 5 was significant, p<0.05, and the effect of individual haplotypes 1 and 31 were suggestive, p<0.10, for DPI 28. The effect of individual haplotypes 5 and 31 were significant, p<0.05, for DPI 42.

The effect of SLA Class I on IFNγ level by day was not significant for DPI 28 or DPI 42. Days post inoculation 7 and DPI 14 were not included in this study since the level of IFNγ was undetectable. There are no individual haplotypes on either day that were significant (data not shown).

The effect of SLA Class II on IL-8 level by day was not significant for DPI 7, DPI 14, DPI 28, or DPI 42. There were no effects of individual haplotypes that were significant (data not shown).

The effect of SLA Class II on IL-1b level was significant for DPI 7, DPI 14, DPI 28, and DPI 42 (Table 13). The effect of individual haplotypes 0.23 and 0.25 on IL-1b level were significant, p<0.05, and the effect of individual haplotype 0.26 was suggestive, p<0.10, for DPI 7. The effect of individual haplotypes 0.23, 0.25, and 0.26 on IL-1b level were all significant, p<0.05, for DPI 14. The effect of individual haplotypes 0.4, 0.23, 0.25, and 0.26 on IL-1b level were all significant, p<0.05 for DPI 28. The effect of individual allele 0.25 on IL-1b was significant, p<0.05, and the effect of individual alleles 0.4 and 0.23 were suggestive, p<0.10, for DPI 42.
The effect of SLA Class II on IFNγ was significant for DPI 42, but was not significant for DPI 28 (Supplementary Materials). Once again, DPI 7 and DPI 14 were not included in this study due to undetectable levels of IFNγ. The effect of individual haplotypes 0.4 and 0.25 on IFNγ level were significant, p<0.05, for DPI 42.

**Discussion**

The analysis of viral and SNA levels, when the area under the curve of the log and log base 2 are computed, respectively, indicates that the SLA Class I haplotypes have some sort of effect on viral and SNA levels (Table 3). However, due to the low number of animals used in this study it is problematic to accurately estimate the effects of the individual haplotypes. Despite this limitation, there is evidence that SLA Class I haplotypes may be associated with viral and SNA levels.

The analysis of viral and SNA levels, when once again using area under the curve of the log and log base 2, respectively, indicates that the SLA Class II haplotypes have some sort of effect on viral and SNA levels (Table 5). There are individual haplotypes that are suggestive, but nothing that is significant. However, there appears to be some significant effect of SLA Class II haplotypes on viral and SNA levels even with the limitation of the low number of animals.

Analysis of IL8, IL1b, and IFNγ levels, when area under the curve of the log is computed, indicates that SLA Class I haplotypes have an effect on IL1b, suggest a possible effect on IL8, and have no effect on IFNγ (Table 4). The individual haplotypes 30 and 31 indicate significant effects, but the accuracy of the individual haplotypes remains problematic due to low animal numbers. This indicates that there is an effect of
SLA Class I haplotypes on IL1b and a possible effect of these haplotypes on IL8 despite limitations.

Analysis of IL8, IL1b, and IFNγ levels, when area under the curve of the log is computed, indicates that SLA Class II haplotypes have an effect on IL1b and IFNγ levels, but no effect on IL8 levels (Table 6). There are individual haplotypes that show suggestive effects and 3 individual haplotypes that show a significant effect for IL1b, haplotypes 0.23, 0.25, and 0.26. The ability to accurately calculate these effects is limited due to the low number of animals. This indicates that there is an effect of SLA Class II haplotypes on IL1b and IFNγ and that we see possible individual haplotype effects that are significant, for IL1b at least.

Analysis of viral, SNA, IL8, IL1b, and IFNγ levels was done using the genotypes of the SLA haplotypes and this style analysis combined certain haplotypes due to them having the same genotype and this could possibly increase the power of the statistical analyses due to having more individuals.

Analysis of viral and SNA levels indicates that Class I SLA 2 genotypes, when area under the curve of the log and log base 2, respectively, was computed, have an effect on viral and SNA levels (Table 7). The individual genotypes w09XX, 10XX, and 12XX have significant effects on SNA levels, and the genotype 02XX has a significant effect on viral levels. However, the accuracy of these results is once again limited by the low number of animals. Despite the limitation, these results indicate that moving to particular genotypes allows us to see more effects than we could with haplotypes.

Continued analysis of viral and SNA levels indicates that Class II DRB1 genotypes, when area under the curve of the log and log base 2, respectively, are
computed, have an effect on viral and SNA levels (Table 8). The individual genotypes 04XX, 06XX, and 11XX all have significant effects on viral levels and the individual genotypes 01XX, 04XX, 10XX, and 11XX all have significant effects on SNA levels. This indicates that we have found some genetic components that are possibly involved in response to infection of the PRRS virus.

Analysis of IL8, IL1b, and IFN\(\gamma\) levels, when area under the curve of the log is computed, indicates that Class II DRB1 genotypes have an effect on IL1b levels but not IL8 or IFN\(\gamma\) levels (Table 9). The individual genotypes 04XX, 10XX, 11XX, and 13XX have significant effects on IL1b levels, although the accuracy of the estimation these effects is limited due to the low number of animals. This indicates that we have found some genetic components that are possibly involved with IL1b involvement with PRRSv infection.

Analysis of viral and SNA levels, when area under the curve of the log and log base 2, respectively, are computed, indicates that Class II DQB1 genotypes have an effect on viral levels but not SNA levels. The individual genotypes 03XX and 07XX have significant effects on viral level but the accuracy of our estimations is limited due to the low number of animals. This indicates that there is involvement of DQB1 genotypes with viral levels despite the limitations involved.

Analysis of IL8, IL1b, and IFN\(\gamma\), when area under the curve of the log is computed, indicates that Class II DQB1 genotypes have an effect on IL1b and IFN\(\gamma\) but not IL8 (Table 11). The individual genotypes 02XX, 03XX, 04XX, and 06XX have significant effects on both IL1b and IFN\(\gamma\) levels and genotype 09XX has a significant effect on IFN\(\gamma\) levels. Our ability to accurately estimate the effects of genotypes 03XX
and 09XX is limited due to the low number of animals with these genotypes. These results indicate that we have found some possible genetic components connected the IL1b and IFNγ involvement with PRRSV infection.

Analysis of viral, SNA, IL8, IL1b, and IFNγ levels computed using log or log base 2 in the case of SNA by day indicates that SLA Class I haplotypes have day effects on viral levels (Table 12) and SLA Class II haplotypes have day effects on IL1b (Table 13). The individual days of DPI 7 and DPI 14 for viral levels have significant SLA Class I haplotype effects but DPI 28 and DPI 42 do not. All the days (DPI 7, 14, 28, and 42) are significant for IL1b levels for SLA Class II haplotype effects. The same individual haplotypes are at least suggestive for all days (haplotypes 0.23, 0.25, and 0.26) with a new haplotype becoming at least suggestive for DPI 28 and DPI 42 (haplotype 0.4). This indicates that there is some haplotype effect on day for viral and IL1b levels in terms of SLA Class I and SLA Class II haplotypes, respectively.

These results indicate that there are definite SLA haplotype and genotype effects on viral, SNA, IL8, IL1b, and IFNγ levels. It is important to keep in mind that the specific effects of these SLA haplotypes and genotypes may not be accurately estimated in the present study due the limited number of available animals. However, We now have a basis for more in depth analysis of SLA haplotype and genotype involvement with PRRSV infection and further analysis should be conducted to determine the depth and specificity of this involvement.
References


Figure 1
Table 1 – SLA class I haplotypes and corresponding allele group specificities identified in Big Pig

<table>
<thead>
<tr>
<th>SLA Class I Haplotypes¹</th>
<th>Specificity</th>
<th>SLA-1</th>
<th>SLA-3</th>
<th>SLA-2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lr-1.0</strong></td>
<td>01XX</td>
<td>01XX</td>
<td>01XX</td>
<td></td>
</tr>
<tr>
<td><strong>Lr-4.0</strong></td>
<td>04XX</td>
<td>04XX/hb06</td>
<td>04XX</td>
<td></td>
</tr>
<tr>
<td><strong>Lr-5.0</strong></td>
<td>04XX</td>
<td>05XX</td>
<td>w08XX</td>
<td></td>
</tr>
<tr>
<td><strong>Lr-6.0</strong></td>
<td>08XX</td>
<td>06XX</td>
<td>05XX</td>
<td></td>
</tr>
<tr>
<td><strong>Lr-21.0</strong></td>
<td>rh03</td>
<td>06XX</td>
<td>05XX</td>
<td></td>
</tr>
<tr>
<td><strong>Lr-22.0</strong></td>
<td>08XX</td>
<td>06XX</td>
<td>12XX</td>
<td></td>
</tr>
<tr>
<td><strong>Lr-25.0</strong></td>
<td>11XX</td>
<td>03XX</td>
<td>07XX</td>
<td></td>
</tr>
<tr>
<td><strong>Lr-27.0</strong></td>
<td>06XX, 08XX</td>
<td>01XX</td>
<td>01XX</td>
<td></td>
</tr>
<tr>
<td><strong>Lr-28.0</strong></td>
<td>09XX, 15XX</td>
<td>07XX</td>
<td>05XX</td>
<td></td>
</tr>
<tr>
<td><strong>Lr-29.0</strong></td>
<td>Blank</td>
<td>05XX</td>
<td>w09XX</td>
<td></td>
</tr>
<tr>
<td><strong>Lr-30.0</strong></td>
<td>07XX</td>
<td>01XX</td>
<td>01XX</td>
<td></td>
</tr>
<tr>
<td><strong>Lr-31.0</strong></td>
<td>Blank</td>
<td>07XX</td>
<td>01XX</td>
<td></td>
</tr>
<tr>
<td><strong>Lr-32.0</strong></td>
<td>07XX</td>
<td>04XX/hb06</td>
<td>02XX</td>
<td></td>
</tr>
<tr>
<td><strong>Lr-33.0²</strong></td>
<td>Blank or 01XX</td>
<td>05XX</td>
<td>06XX</td>
<td></td>
</tr>
<tr>
<td><strong>Lr-34.0</strong></td>
<td>Blank</td>
<td>04XX/hb06</td>
<td>05XX</td>
<td></td>
</tr>
<tr>
<td><strong>Lr-39.0</strong></td>
<td>Blank</td>
<td>05XX</td>
<td>10XX</td>
<td></td>
</tr>
</tbody>
</table>

¹ Low-resolution (Lr) haplotype
² Ambiguous SLA-1 specificity due to the identification of this haplotype in only one animal
<table>
<thead>
<tr>
<th>SLA Class II Haplotypes&lt;sup&gt;1&lt;/sup&gt;</th>
<th>DRB1</th>
<th>DQB1</th>
<th>DQA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lr-0.1</td>
<td>01XX/be01/ha04</td>
<td>01XX</td>
<td>01XX</td>
</tr>
<tr>
<td>Lr-0.2</td>
<td>02XX</td>
<td>02XX</td>
<td>02XX</td>
</tr>
<tr>
<td>Lr-0.4</td>
<td>02XX</td>
<td>04XX</td>
<td>02XX</td>
</tr>
<tr>
<td>Lr-0.12</td>
<td>06XX</td>
<td>07XX</td>
<td>01XX</td>
</tr>
<tr>
<td>Lr-0.13</td>
<td>04XX</td>
<td>03XX</td>
<td>02XX</td>
</tr>
<tr>
<td>Lr-0.15b</td>
<td>04XX</td>
<td>02XX</td>
<td>02XX</td>
</tr>
<tr>
<td>Lr-0.19a</td>
<td>04XX</td>
<td>07XX</td>
<td>03XX</td>
</tr>
<tr>
<td>Lr-0.22</td>
<td>06XX&lt;sup&gt;2&lt;/sup&gt;</td>
<td>02XX</td>
<td>02XX</td>
</tr>
<tr>
<td>Lr-0.23</td>
<td>10XX/er01</td>
<td>06XX/zs12</td>
<td>01XX</td>
</tr>
<tr>
<td>Lr-0.24</td>
<td>07XX</td>
<td>02XX</td>
<td>02XX</td>
</tr>
<tr>
<td>Lr-0.25</td>
<td>13XX</td>
<td>09XX</td>
<td>04XX + w05XX</td>
</tr>
<tr>
<td>Lr-0.26</td>
<td>11XX</td>
<td>04XX</td>
<td>02XX</td>
</tr>
</tbody>
</table>

<sup>1</sup> Low-resolution (Lr) haplotype

<sup>2</sup> Also positive with the medium-resolution primer pair specific for polymorphisms of DRB1*0401~02/04ga01/04ta01 at +160 and +243.
Table 3 – Effect of SLA Class I Haplotype on Virus and Serum Neutralizing Antibody (SNA) Level

<table>
<thead>
<tr>
<th>Lr</th>
<th>N#</th>
<th>Virus¹</th>
<th>SNA²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>50.61 ± 25.18</td>
<td>11.93 ± 9.66</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td><strong>80.12± 29.52</strong></td>
<td>-12.15 ± 11.66</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>-50.30 ± 66.83</td>
<td>15.06 ± 25.22</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>40.72 ± 46.90</td>
<td>21.43 ± 17.91</td>
</tr>
<tr>
<td>21</td>
<td>3</td>
<td>-91.24 ± 65.78</td>
<td>13.98 ± 24.76</td>
</tr>
<tr>
<td>22</td>
<td>35</td>
<td>57.43 ± 26.40</td>
<td>-24.38 ± 10.14</td>
</tr>
<tr>
<td>25</td>
<td>1</td>
<td>125.22 ± 111.09</td>
<td>-41.14 ± 41.82</td>
</tr>
<tr>
<td>27</td>
<td>1</td>
<td>59.96 ± 113.33</td>
<td>33.50 ± 42.76</td>
</tr>
<tr>
<td>28</td>
<td>11</td>
<td>39.41 ± 42.82</td>
<td>5.04 ± 16.82</td>
</tr>
<tr>
<td>29</td>
<td>6</td>
<td>109.58 ± 51.56</td>
<td>47.11 ± 21.12</td>
</tr>
<tr>
<td>30</td>
<td>4</td>
<td><strong>-383.40± 59.04</strong></td>
<td>-44.09 ± 22.33</td>
</tr>
<tr>
<td>31</td>
<td>3</td>
<td>140.14 ± 68.79</td>
<td>-23.32 ± 26.08</td>
</tr>
<tr>
<td>32</td>
<td>1</td>
<td><strong>-328.60± 116.53</strong></td>
<td>7.74 ± 44.08</td>
</tr>
<tr>
<td>33</td>
<td>1</td>
<td>126.83 ± 111.09</td>
<td>28.86 ± 41.82</td>
</tr>
<tr>
<td>34</td>
<td>1</td>
<td>-29.33 ± 111.63</td>
<td>-4.84 ± 42.02</td>
</tr>
<tr>
<td>39</td>
<td>22</td>
<td>55.85 ± 33.02</td>
<td>-34.72 ± 12.92</td>
</tr>
</tbody>
</table>

¹Total number of times haplotype was observed in analysis of virus and SNA
²SLA haplotype effect on virus level ± standard error; difference from the mean (µ=168.9 log of parts/ml)
³SLA haplotype effect on SNA level ± standard error; difference from the mean (µ=88.9 log base 2 of dilution)
⁴Significant Effect p<0.05
<table>
<thead>
<tr>
<th>Lr</th>
<th>N#</th>
<th>IL8(^1)</th>
<th>IL1b(^2)</th>
<th>IFN(^\gamma)(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>5.45 ± 11.03</td>
<td>-217.07(^B) ± 79.38</td>
<td>-1.31 ± 1.57</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>8.68 ± 13.09</td>
<td>-98.19 ± 94.23</td>
<td>-3.60 ± 1.81</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>10.34 ± 25.31</td>
<td>379.03 ± 182.17</td>
<td>-7.50 ± 3.48</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>28.76 ± 20.24</td>
<td>-158.18 ± 145.66</td>
<td>4.99 ± 3.77</td>
</tr>
<tr>
<td>21</td>
<td>1</td>
<td>-29.19 ± 34.74</td>
<td>-134.55 ± 250.05</td>
<td>-3.10 ± 4.73</td>
</tr>
<tr>
<td>22</td>
<td>18</td>
<td>8.34 ± 10.06</td>
<td>-101.16 ± 72.41</td>
<td>-0.56 ± 1.39</td>
</tr>
<tr>
<td>25</td>
<td>1</td>
<td>21.92 ± 34.74</td>
<td>-134.55 ± 250.05</td>
<td>-3.10 ± 4.73</td>
</tr>
<tr>
<td>27</td>
<td>1</td>
<td>67.08 ± 36.03</td>
<td>-233.65 ± 259.35</td>
<td>-3.15 ± 4.96</td>
</tr>
<tr>
<td>28</td>
<td>3</td>
<td>-17.31 ± 21.32</td>
<td>250.05 ± 153.43</td>
<td>-1.59 ± 2.93</td>
</tr>
<tr>
<td>30</td>
<td>2</td>
<td>-95.04(^A) ± 25.03</td>
<td>43.92 ± 180.12</td>
<td>-4.60 ± 3.41</td>
</tr>
<tr>
<td>31</td>
<td>2</td>
<td>30.53 ± 25.21</td>
<td>517.85(^A) ± 181.47</td>
<td>4.44 ± 4.73</td>
</tr>
<tr>
<td>34</td>
<td>1</td>
<td>-29.48 ± 34.99</td>
<td>-200.23 ± 251.84</td>
<td>-3.85 ± 4.77</td>
</tr>
<tr>
<td>39</td>
<td>11</td>
<td>-10.08 ± 15.30</td>
<td>32.74 ± 110.15</td>
<td>-1.26 ± 2.10</td>
</tr>
</tbody>
</table>

\(^\#\)Total number of times haplotype was observed in analysis of IL8, IL1b, and IFN\(^\gamma\)

\(^1\)SLA haplotype effect on IL8 level ± standard error; difference from the mean\((\mu=70.5 \log \text{ of pg/ml})\)

\(^2\)SLA haplotype effect on IL1b level ± standard error; difference from the mean\((\mu=84.1 \log \text{ of pg/ml})\)

\(^3\)SLA haplotype effect on IFN\(^\gamma\) level ± standard error; difference from the mean\((\mu=53.3 \log \text{ of pg/ml})\)

\(^A\)Significant Effect p<0.05

\(^B\)Suggestive Effect p<0.10
### Table 5 – Effect of SLA Class II Haplotype on Virus and Serum Neutralizing Antibody (SNA) Level

<table>
<thead>
<tr>
<th>Lr</th>
<th>N#</th>
<th>Virus¹</th>
<th>SNA²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>31</td>
<td>-35.72 ± 30.72</td>
<td>-24.73B ± 10.56</td>
</tr>
<tr>
<td>0.2</td>
<td>13</td>
<td>17.94 ± 40.51</td>
<td>-9.58 ± 14.37</td>
</tr>
<tr>
<td>0.4</td>
<td>32</td>
<td>37.95 ± 38.55</td>
<td>-3.95 ± 13.39</td>
</tr>
<tr>
<td>0.12</td>
<td>4</td>
<td>-255.86A ± 65.13</td>
<td>-11.09 ± 21.49</td>
</tr>
<tr>
<td>0.13</td>
<td>4</td>
<td>119.83 ± 67.61</td>
<td>4.06 ± 22.59</td>
</tr>
<tr>
<td>0.15b</td>
<td>31</td>
<td>46.19 ± 29.92</td>
<td>-21.20 ± 10.14</td>
</tr>
<tr>
<td>0.19</td>
<td>1</td>
<td>86.64 ± 125.75</td>
<td>-30.51 ± 41.49</td>
</tr>
<tr>
<td>0.22</td>
<td>1</td>
<td>-154.69 ± 125.75</td>
<td>46.49 ± 41.49</td>
</tr>
<tr>
<td>0.23</td>
<td>24</td>
<td>36.60 ± 32.18</td>
<td>-28.18B ± 11.00</td>
</tr>
<tr>
<td>0.24</td>
<td>6</td>
<td>84.74 ± 58.23</td>
<td>33.97 ± 21.50</td>
</tr>
<tr>
<td>0.25</td>
<td>3</td>
<td>-57.03 ± 74.38</td>
<td>14.84 ± 24.50</td>
</tr>
<tr>
<td>0.26</td>
<td>22</td>
<td>73.22 ± 36.43</td>
<td>-29.87B ± 12.80</td>
</tr>
</tbody>
</table>

# Total number of times haplotype was observed in analysis of virus and SNA
¹SLA haplotype effect on virus level ± standard error; difference from the mean (µ=168.9 log of parts/ml)
²SLA haplotype effect on SNA level ± standard error; difference from the mean (µ=88.9 log base 2 of dilution)
A Significant Effect p<0.05
B Suggestive Effect p<0.10
Table 6 – Effect of SLA Class II Haplotype on IL8, IL1b, and IFNγ Level

<table>
<thead>
<tr>
<th>Lr</th>
<th>N#</th>
<th>IL8&lt;sup&gt;1&lt;/sup&gt; ± SE</th>
<th>IL1b&lt;sup&gt;2&lt;/sup&gt; ± SE</th>
<th>IFNG&lt;sup&gt;3&lt;/sup&gt; ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>15</td>
<td>-23.34 ± 12.33</td>
<td>-35.70 ± 67.79</td>
<td>0.37 ± 1.30</td>
</tr>
<tr>
<td>0.2</td>
<td>5</td>
<td>-2.07 ± 18.07</td>
<td>-4.20 ± 99.30</td>
<td>-1.98 ± 1.92</td>
</tr>
<tr>
<td>0.4</td>
<td>17</td>
<td>5.31 ± 15.04</td>
<td>-142.43 ± 82.63</td>
<td>-3.99&lt;sup&gt;B&lt;/sup&gt; ± 1.61</td>
</tr>
<tr>
<td>0.12</td>
<td>2</td>
<td>-40.29 ± 27.01</td>
<td>-99.05 ± 148.46</td>
<td>-3.03 ± 2.85</td>
</tr>
<tr>
<td>0.13</td>
<td>2</td>
<td>49.05 ± 27.09</td>
<td>-98.52 ± 148.87</td>
<td>7.81&lt;sup&gt;B&lt;/sup&gt; ± 2.86</td>
</tr>
<tr>
<td>0.15b</td>
<td>16</td>
<td>-0.95 ± 10.61</td>
<td>-104.61 ± 58.32</td>
<td>-1.21 ± 1.12</td>
</tr>
<tr>
<td>0.19</td>
<td>1</td>
<td>10.18 ± 37.48</td>
<td>-138.52 ± 206.02</td>
<td>-0.67 ± 3.96</td>
</tr>
<tr>
<td>0.23</td>
<td>10</td>
<td>-17.58 ± 11.90</td>
<td>325.95&lt;sup&gt;A&lt;/sup&gt; ± 65.43</td>
<td>-1.89 ± 1.43</td>
</tr>
<tr>
<td>0.25</td>
<td>2</td>
<td>6.78 ± 26.69</td>
<td>506.04&lt;sup&gt;A&lt;/sup&gt; ± 146.67</td>
<td>6.63&lt;sup&gt;B&lt;/sup&gt; ± 0.82</td>
</tr>
<tr>
<td>0.26</td>
<td>12</td>
<td>12.91 ± 14.10</td>
<td>208.95&lt;sup&gt;A&lt;/sup&gt; ± 77.48</td>
<td>-2.05 ± 1.50</td>
</tr>
</tbody>
</table>

<sup>#</sup>Total number of times haplotype was observed in analysis of IL8, IL1b, and IFNγ

<sup>1</sup>SLA haplotype effect on IL8 level ± standard error; difference from the mean ($\mu=70.5$ log of pg/ml)

<sup>2</sup>SLA haplotype effect on IL1b level ± standard error; difference from the mean ($\mu=84.1$ log of pg/ml)

<sup>3</sup>SLA haplotype effect on IFNγ level ± standard error; difference from the mean ($\mu=53.3$ log of pg/ml)

<sup>A</sup>Significant Effect $p<0.05$

<sup>B</sup>Suggestive Effect $p<0.10$
Table 7 – Class I SLA-2 Locus Effect on Virus and Serum Neutralizing Antibody (SNA) Level

<table>
<thead>
<tr>
<th>Allele</th>
<th>N#</th>
<th>Virus¹</th>
<th>SNA²</th>
</tr>
</thead>
<tbody>
<tr>
<td>01XX</td>
<td>58</td>
<td>-6.88 ± 34.57</td>
<td>5.60 ± 10.70</td>
</tr>
<tr>
<td>02XX</td>
<td>1</td>
<td>-358.15 ± 137.02</td>
<td>-1.35 ± 41.70</td>
</tr>
<tr>
<td>04XX</td>
<td>40</td>
<td>19.05 ± 36.15</td>
<td>-14.46 ± 11.24</td>
</tr>
<tr>
<td>05XX</td>
<td>23</td>
<td>5.76 ± 45.39</td>
<td>13.82 ± 14.10</td>
</tr>
<tr>
<td>06XX</td>
<td>1</td>
<td>121.12 ± 133.87</td>
<td>34.87 ± 40.70</td>
</tr>
<tr>
<td>07XX</td>
<td>1</td>
<td>119.51 ± 133.87</td>
<td>-35.13 ± 40.70</td>
</tr>
<tr>
<td>w08XX</td>
<td>3</td>
<td>-79.39 ± 81.67</td>
<td>11.93 ± 24.91</td>
</tr>
<tr>
<td>w09XX</td>
<td>6</td>
<td>106.17B ± 61.72</td>
<td>40.87A ± 20.16</td>
</tr>
<tr>
<td>10XX</td>
<td>22</td>
<td>45.22 ± 40.74</td>
<td>-32.76A ± 12.75</td>
</tr>
<tr>
<td>12XX</td>
<td>35</td>
<td>27.58 ± 34.66</td>
<td>-23.39A ± 10.79</td>
</tr>
</tbody>
</table>

#Total number of times allele was observed in analysis of virus and SNA
¹ Allele effect on virus level ± standard error; difference from the mean (µ=168.9 log of parts/ml)
² Allele effect on SNA level ± standard error; difference from the mean (µ=88.9 log base 2 of dilution)
A Significant Effect p<0.05
B Suggestive Effect p<0.10
Table 8 – Class II DRB1 Effect on Virus and Serum Neutralizing Antibody (SNA) Level

<table>
<thead>
<tr>
<th>Allele</th>
<th>N#</th>
<th>Virus 1</th>
<th>SNA 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>01XX</td>
<td>31</td>
<td>-33.49 ± 27.15</td>
<td>-25.89A ± 9.46</td>
</tr>
<tr>
<td>02XX</td>
<td>46</td>
<td>41.88 ± 25.33</td>
<td>-5.49 ± 8.65</td>
</tr>
<tr>
<td>04XX</td>
<td>35</td>
<td>56.89A ± 25.73</td>
<td>-18.34A ± 8.91</td>
</tr>
<tr>
<td>06XX</td>
<td>5</td>
<td>-230.99A ± 54.33</td>
<td>0.11 ± 18.12</td>
</tr>
<tr>
<td>07XX</td>
<td>6</td>
<td>97.59B ± 50.56</td>
<td>32.86B ± 18.28</td>
</tr>
<tr>
<td>10XX</td>
<td>24</td>
<td>38.74 ± 28.70</td>
<td>-29.60A ± 9.89</td>
</tr>
<tr>
<td>11XX</td>
<td>22</td>
<td>79.33A ± 30.54</td>
<td>30.64A ± 11.17</td>
</tr>
<tr>
<td>13XX</td>
<td>3</td>
<td>-49.94 ± 68.97</td>
<td>15.71 ± 22.91</td>
</tr>
</tbody>
</table>

#Total number of times allele was observed in analysis of virus and SNA

1 Allele effect on virus level ± standard error; difference from the mean (μ=168.9 log of parts/ml)

2 Allele effect on SNA level ± standard error; difference from the mean (μ=88.9 log base 2 of dilution)

A Significant Effect p<0.05

B Suggestive Effect p<0.10

Table 9 – Class II DRB1 Effect on IL8, IL1b, and IFNγ Level

<table>
<thead>
<tr>
<th>Allele</th>
<th>N#</th>
<th>IL8 1</th>
<th>IL1b 2</th>
<th>IFNγ 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>01XX</td>
<td>15</td>
<td>-17.38 ± 11.94</td>
<td>-6.49 ± 13.38</td>
<td>0.70 ± 1.36</td>
</tr>
<tr>
<td>02XX</td>
<td>22</td>
<td>13.29 ± 10.71</td>
<td>-18.48 ± 12.01</td>
<td>-2.39B ± 1.25</td>
</tr>
<tr>
<td>04XX</td>
<td>19</td>
<td>11.15 ± 10.07</td>
<td>-33.83A ± 11.28</td>
<td>-0.10 ± 1.15</td>
</tr>
<tr>
<td>06XX</td>
<td>2</td>
<td>-36.10 ± 25.51</td>
<td>-18.94 ± 28.59</td>
<td>-2.93 ± 2.91</td>
</tr>
<tr>
<td>10XX</td>
<td>10</td>
<td>-10.73 ± 11.16</td>
<td>51.43A ± 12.51</td>
<td>-1.52 ± 1.46</td>
</tr>
<tr>
<td>11XX</td>
<td>12</td>
<td>24.11B ± 12.70</td>
<td>-55.73A ± 14.23</td>
<td>-0.73 ± 1.46</td>
</tr>
<tr>
<td>13XX</td>
<td>2</td>
<td>15.66 ± 25.28</td>
<td>82.04A ± 28.34</td>
<td>6.97A ± 2.88</td>
</tr>
</tbody>
</table>

#Total number of times allele was observed in analysis of IL8, IL1b, and IFNγ

1 Allele effect on IL8 level ± standard error; difference from the mean (μ=70.5 log of pg/ml)

2 Allele effect on IL1b level ± standard error; difference from the mean (μ=84.1 log of pg/ml)

3 Allele effect on IFNγ level ± standard error; difference from the mean (μ=53.3 log of pg/ml)

A Significant effect p<0.05

B Suggestive effect p<0.10
### Table 10 – Class II DQB1 Effect on Virus and Serum Neutralizing Antibody (SNA) Level

<table>
<thead>
<tr>
<th>Allele</th>
<th>N(^a)</th>
<th>Virus(^1)</th>
<th>SNA(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01XX</td>
<td>31</td>
<td>-29.63 ± 28.36</td>
<td>-15.56 ± 9.89</td>
</tr>
<tr>
<td>02XX</td>
<td>51</td>
<td>44.24(^B) ± 24.19</td>
<td>-5.26 ± 8.46</td>
</tr>
<tr>
<td>03XX</td>
<td>4</td>
<td>133.71(^A) ± 62.00</td>
<td>21.23 ± 21.25</td>
</tr>
<tr>
<td>04XX</td>
<td>54</td>
<td>54.47(^B) ± 31.12</td>
<td>15.47 ± 11.13</td>
</tr>
<tr>
<td>06XX</td>
<td>24</td>
<td>37.01 ± 30.61</td>
<td>-21.51(^A) ± 10.71</td>
</tr>
<tr>
<td>07XX</td>
<td>5</td>
<td>-182.43(^A) ± 55.82</td>
<td>-10.91 ± 19.11</td>
</tr>
<tr>
<td>09XX</td>
<td>3</td>
<td>-57.37 ± 70.77</td>
<td>16.55 v 24.17</td>
</tr>
</tbody>
</table>

\(^a\)Total number of times allele was observed in analysis of virus and SNA

\(^1\)Allele effect on virus level ± standard error; difference from the mean (\(\mu = 168.9\) log of parts/ml)

\(^2\)Allele effect on SNA level ± standard error; difference from the mean (\(\mu = 88.9\) log base 2 of dilution)

\(^A\)Significant Effect p<0.05

\(^B\)Suggestive Effect p<0.10
Table 11 – Class II DQB1 Effect on IL8, IL1b, and IFNγ Level

<table>
<thead>
<tr>
<th>Allele</th>
<th>N#</th>
<th>IL8 (^1)</th>
<th>IL1b (^2)</th>
<th>IFNγ (^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01XX</td>
<td>15</td>
<td>-23.04(^A) ± 11.31</td>
<td>-5.17 ± 13.94</td>
<td>-0.50 ± 1.21</td>
</tr>
<tr>
<td>02XX</td>
<td>21</td>
<td>-1.66 ± 9.71</td>
<td>-24.62(^A) ± 11.98</td>
<td>-2.26(^A) ± 1.05</td>
</tr>
<tr>
<td>03XX</td>
<td>2</td>
<td>48.92(^B) ± 24.88</td>
<td>-39.80 ± 30.67</td>
<td>7.00(^A) ± 2.65</td>
</tr>
<tr>
<td>04XX</td>
<td>29</td>
<td>10.70 ± 12.70</td>
<td>-40.12(^A) ± 15.66</td>
<td>-3.78(^A) ± 1.38</td>
</tr>
<tr>
<td>06XX</td>
<td>10</td>
<td>-17.50 ± 11.05</td>
<td>53.91(^A) ± 13.63</td>
<td>-2.90(^A) ± 1.33</td>
</tr>
<tr>
<td>07XX</td>
<td>3</td>
<td>-22.83 ± 20.41</td>
<td>-35.26 ± 25.17</td>
<td>-2.95 ± 2.18</td>
</tr>
<tr>
<td>09XX</td>
<td>2</td>
<td>5.40 ± 24.64</td>
<td>91.05(^A) ± 30.37</td>
<td>5.39(^A) ± 2.63</td>
</tr>
</tbody>
</table>

\(^A\)Total number of times allele was observed in analysis of IL8, IL1b, and IFNγ

\(^1\)Allele effect on IL8 level ± standard error; difference from the mean (μ=70.5 log of pg/ml)

\(^2\)Allele effect on IL1b level ± standard error; difference from the mean (μ=84.1 log of pg/ml)

\(^3\)Allele effect on IFNγ level ± standard error; difference from the mean (μ=53.3 log of pg/ml)

\(^A\)Significant effect p<0.05

\(^B\)Suggestive Effect p<0.10
Table 12 – Effect of SLA Class I Haplotype within Day on Virus Level

<table>
<thead>
<tr>
<th>Day</th>
<th>Lr</th>
<th>N#</th>
<th>$7^2$</th>
<th>$14^3$</th>
<th>$28^4$</th>
<th>$42^5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>0.363 ± 0.392</td>
<td>0.992 ± 0.496</td>
<td>-0.270 ± 0.689</td>
<td>0.323 ± 0.759</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>-0.004 ± 0.460</td>
<td>1.512B ± 0.582</td>
<td>0.279 ± 0.808</td>
<td>0.456 ± 0.889</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>-3.910A ± 1.040</td>
<td>-0.538 ± 1.317</td>
<td>-0.607 ± 1.828</td>
<td>-0.744 ± 2.013</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>1.144 ± 0.730</td>
<td>0.616 ± 0.924</td>
<td>0.158 ± 1.283</td>
<td>0.720 ± 1.413</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>3</td>
<td>0.156 ± 1.024</td>
<td>-1.718 ± 1.296</td>
<td>1.144 ± 1.800</td>
<td>-2.188 ± 1.981</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>35</td>
<td>0.383 ± 0.411</td>
<td>1.302B ± 0.520</td>
<td>-0.726 ± 0.722</td>
<td>-0.420 ± 0.795</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>1</td>
<td>1.595 ± 1.730</td>
<td>2.984 ± 2.189</td>
<td>-1.511 ± 3.040</td>
<td>-2.391 ± 3.346</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>1</td>
<td>0.860 ± 1.764</td>
<td>0.680 ± 2.233</td>
<td>4.397 ± 3.101</td>
<td>-1.964 ± 3.414</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>11</td>
<td>0.847 ± 0.667</td>
<td>0.595 ± 0.844</td>
<td>-0.380 ± 1.172</td>
<td>1.406 ± 1.290</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>6</td>
<td>1.538 ± 0.803</td>
<td>2.385B ± 1.016</td>
<td>-1.517 ± 1.411</td>
<td>-0.356 ± 1.553</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>4</td>
<td>-6.410A ± 0.919</td>
<td>-7.168A ± 1.163</td>
<td>-1.072 ± 1.615</td>
<td>0.270 ± 1.778</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>3</td>
<td>0.913 ± 1.071</td>
<td>2.104 ± 1.356</td>
<td>1.659 ± 1.882</td>
<td>2.909 ± 2.072</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>1</td>
<td>2.037 ± 1.730</td>
<td>2.696 ± 2.189</td>
<td>0.396 ± 3.040</td>
<td>-2.391 ± 3.346</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>1</td>
<td>1.761 ± 1.738</td>
<td>-1.748 ± 2.200</td>
<td>-0.644 ± 3.054</td>
<td>7.890 ± 3.362</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>22</td>
<td>0.944 ± 0.514</td>
<td>1.005 ± 0.651</td>
<td>0.070 ± 0.903</td>
<td>-0.105 ± 0.994</td>
<td></td>
</tr>
</tbody>
</table>

#Total number of times haplotype was observed in analysis of virus
1Number of days post inoculation
2SLA haplotype effect on virus level ± standard error; difference from the mean ($\mu=6.89$ log of parts/ml)
3SLA haplotype effect on virus level ± standard error; difference from the mean ($\mu=6.81$ log of parts/ml)
4SLA haplotype effect on virus level ± standard error; difference from the mean ($\mu=4.72$ log of parts/ml)
5SLA haplotype effect on virus level ± standard error; difference from the mean ($\mu=1.02$ log of parts/ml)
A Significant Effect p<0.05
B Suggestive Effect p<0.10
Table 13 – Effect of SLA Class II Haplotype within Day on IL1b Level

<table>
<thead>
<tr>
<th>Day²</th>
<th>7²</th>
<th>14³</th>
<th>28⁴</th>
<th>42⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>15</td>
<td>0.106 ± 0.243</td>
<td>0.107 ± 0.260</td>
<td>-0.197 ± 0.109</td>
</tr>
<tr>
<td>0.2</td>
<td>5</td>
<td>-0.058 ± 0.356</td>
<td>0.125 ± 0.381</td>
<td>-0.036 ± 0.160</td>
</tr>
<tr>
<td>0.4</td>
<td>17</td>
<td>-0.168 ± 0.297</td>
<td>-0.119 ± 0.317</td>
<td>-0.535A ± 0.133</td>
</tr>
<tr>
<td>0.12</td>
<td>2</td>
<td>-0.421 ± 0.533</td>
<td>-0.107 ± 0.570</td>
<td>-0.297 ± 0.239</td>
</tr>
<tr>
<td>0.13</td>
<td>2</td>
<td>-0.235 ± 0.534</td>
<td>-0.547 ± 0.572</td>
<td>-0.122 ± 0.240</td>
</tr>
<tr>
<td>0.15b</td>
<td>16</td>
<td>-0.351 ± 0.209</td>
<td>-0.348 ± 0.224</td>
<td>-0.185 ± 0.094</td>
</tr>
<tr>
<td>0.19</td>
<td>1</td>
<td>-0.747 ± 0.740</td>
<td>-0.877 ± 0.791</td>
<td>-0.067 ± 0.332</td>
</tr>
<tr>
<td>0.23</td>
<td>10</td>
<td>0.918A ± 0.235</td>
<td>1.067A ± 0.251</td>
<td>0.489A ± 0.105</td>
</tr>
<tr>
<td>0.25</td>
<td>2</td>
<td>1.594A ± 0.527</td>
<td>1.477A ± 0.563</td>
<td>1.376A ± 0.236</td>
</tr>
<tr>
<td>0.26</td>
<td>12</td>
<td>-0.637B ± 0.278</td>
<td>-0.778A ± 0.298</td>
<td>-0.425A ± 0.125</td>
</tr>
</tbody>
</table>

*Total number of times haplotype was observed in analysis of IL1b
¹Number of days post inoculation
²SLA haplotype effect on IL1b level ± standard error; difference from the mean (µ=2.53 log of pg/ml)
³SLA haplotype effect on IL1b level ± standard error; difference from the mean (µ=2.58 log of pg/ml)
⁴SLA haplotype effect on IL1b level ± standard error; difference from the mean (µ=2.31 log of pg/ml)
⁵SLA haplotype effect on IL1b level ± standard error; difference from the mean (µ=2.25 log of pg/ml)
A Significant effect p<0.05
B Suggestive effect p<0.10
## Supplementary Material

### Table 1 – SLA Class I and Class II Genotypes of Individual Animal

<table>
<thead>
<tr>
<th>Pig ID</th>
<th>Class I Genotype</th>
<th>Class II Genotype</th>
<th>Pig ID</th>
<th>Class I Genotype</th>
<th>Class II Genotype</th>
<th>Pig ID</th>
<th>Class I Genotype</th>
<th>Class II Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28.0/39.0</td>
<td>39</td>
<td>40</td>
<td>1.0/4.0</td>
<td>0.2/0.4</td>
<td>76</td>
<td>4.0/6.0</td>
<td>0.4/0.12</td>
</tr>
<tr>
<td>2</td>
<td>4.0/30.0</td>
<td>41</td>
<td>1.0/39.0</td>
<td>0.1/0.26</td>
<td>78</td>
<td>22.0/29.0</td>
<td>0.15b/0.24</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4.0/29.0</td>
<td>42</td>
<td>1.0/22.0</td>
<td>0.4/0.15b</td>
<td>79</td>
<td>1.0/39.0</td>
<td>0.23/0.26</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.0/39.0</td>
<td>43</td>
<td>1.0/39.0</td>
<td>0.1/0.26</td>
<td>80</td>
<td>4.0/22.0</td>
<td>0.4/0.15b</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4.0/28.0</td>
<td>44</td>
<td>4.0/21.0</td>
<td>0.4/0.22</td>
<td>81</td>
<td>22.0/39.0</td>
<td>0.15b/0.23</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.0/1.0</td>
<td>45</td>
<td>22.0/22.0</td>
<td>0.15b/0.15b</td>
<td>82</td>
<td>1.0/1.0</td>
<td>0.1/0.26</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1.0/22.0</td>
<td>46</td>
<td>1.0/28.0</td>
<td>0.23/0.26</td>
<td>83</td>
<td>5/39.0</td>
<td>0.1/0.25</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1.0/22.0</td>
<td>47</td>
<td>1.0/1.0</td>
<td></td>
<td>84</td>
<td>4.0/39.0</td>
<td>0.2/0.23</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>4.0/6.0</td>
<td>48</td>
<td>1.0/39.0</td>
<td>0.1/0.1</td>
<td>85</td>
<td>22.0/22.0</td>
<td>0.15b/0.15b</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>29.0/31.0</td>
<td>49</td>
<td>4.0/6.0</td>
<td>0.4/0.23</td>
<td>86</td>
<td>22.0/22.0</td>
<td>0.15b/0.15b</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1.0/39.0</td>
<td>50</td>
<td>5.0/28.0</td>
<td>0.23/0.25</td>
<td>87</td>
<td>22.0/22.0</td>
<td>0.15b/0.15b</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>4.0/6.0</td>
<td>51</td>
<td>4.0/39.0</td>
<td>0.1/0.4</td>
<td>88</td>
<td>4.0/6.0</td>
<td>0.4/0.23</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>1.0/28.0</td>
<td>52</td>
<td>22.0/39.0</td>
<td>0.15b/0.23</td>
<td>89</td>
<td>28.0/39.0</td>
<td>0.23/0.23</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>22.0/39.0</td>
<td>53</td>
<td>4.0/39.0</td>
<td>0.1/0.4</td>
<td>90</td>
<td>4.0/28.0</td>
<td>0.2/0.23</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>0.1/0.4</td>
<td>54</td>
<td>4.0/30.0</td>
<td>0.1/0.4</td>
<td>91</td>
<td>4.0/6.0</td>
<td>0.4/0.13</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>1.0/39.0</td>
<td>55</td>
<td>1.0/30.0</td>
<td>0.1/0.1</td>
<td>92</td>
<td>1.0/25.0</td>
<td>0.4/0.19</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>1.0/39.0</td>
<td>56</td>
<td>22.0/34.0</td>
<td>0.12/0.15b</td>
<td>93</td>
<td>22.0/22.0</td>
<td>0.15b/0.26</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>1.0/39.0</td>
<td>57</td>
<td>4.0/28.0</td>
<td>0.4/0.23</td>
<td>94</td>
<td>4.0/22.0</td>
<td>0.4/0.15b</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>1.0/4.0</td>
<td>58</td>
<td>4.0/6.0</td>
<td>0.4/0.13</td>
<td>95</td>
<td>1.0/22.0</td>
<td>0.15b/0.26</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1.0/4.0</td>
<td>59</td>
<td>31.0/39.0</td>
<td>0.23/0.23</td>
<td>96</td>
<td>1.0/22.0</td>
<td>0.15b/0.26</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>1.0/22.0</td>
<td>60</td>
<td>1.0/1.0</td>
<td>0.1/0.26</td>
<td>97</td>
<td>4.0/39.0</td>
<td>0.1/0.4</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>1.0/1.0</td>
<td>61</td>
<td>27.0/39.0</td>
<td>0.1/0.2</td>
<td>98</td>
<td>1.0/4.0</td>
<td>0.2/0.26</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>0.1/0.15b</td>
<td>62</td>
<td>4.0/30.0</td>
<td>0.1/0.4</td>
<td>99</td>
<td>4.0/39.0</td>
<td>0.4/0.23</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.26/0.26</td>
<td>63</td>
<td>1.0/4.0</td>
<td>0.1/0.4</td>
<td>100</td>
<td>1.0/22.0</td>
<td>0.1/0.15b</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>0.12/0.23</td>
<td>64</td>
<td>4.0/39.0</td>
<td>0.1/0.4</td>
<td>101</td>
<td>4.0/28.0</td>
<td>0.4/0.23</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>0.12/0.23</td>
<td>65</td>
<td>1.0/31.0</td>
<td>0.13/0.26</td>
<td>102</td>
<td>1.0/4.0</td>
<td>0.2/0.26</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>1.0/39.0</td>
<td>66</td>
<td>22.0/22.0</td>
<td>0.15b/0.15b</td>
<td>103</td>
<td>4.0/29.0</td>
<td>0.4/0.24</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>1.0/39.0</td>
<td>67</td>
<td>1.0/21.0</td>
<td>0.12/0.26</td>
<td>104</td>
<td>4.0/22.0</td>
<td>0.4/0.15b</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>1.0/1.0</td>
<td>68</td>
<td>1.0/22.0</td>
<td>0.4/0.15b</td>
<td>105</td>
<td>1.0/6.0</td>
<td>0.4/0.13</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>0.1/0.15b</td>
<td>69</td>
<td>22.0/22.0</td>
<td>0.4/0.23</td>
<td>106</td>
<td>4.0/6.0</td>
<td>0.4/0.23</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>0.1/0.26</td>
<td>70</td>
<td>4.0/22.0</td>
<td>0.4/0.15b</td>
<td>107</td>
<td>4.0/22.0</td>
<td>0.15b/0.24</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>1.0/22.0</td>
<td>71</td>
<td>4.0/6.0</td>
<td>0.4/0.23</td>
<td>108</td>
<td>1.0/39.0</td>
<td>0.1/0.23</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>0.1/0.26</td>
<td>72</td>
<td>1.0/4.0</td>
<td>0.2/0.4</td>
<td>109</td>
<td>22.0/22.0</td>
<td>0.15b/0.15b</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>4.0/5.0</td>
<td>73</td>
<td>1.0/22.0</td>
<td>0.1/0.15b</td>
<td>110</td>
<td>22.0/22.0</td>
<td>0.15b/0.15b</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>4.0/5.0</td>
<td>74</td>
<td>1.0/22.0</td>
<td>0.1/0.15b</td>
<td>111</td>
<td>22.0/22.0</td>
<td>0.15b/0.15b</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>4.0/5.0</td>
<td>75</td>
<td>1.0/22.0</td>
<td>0.1/0.15b</td>
<td>112</td>
<td>22.0/22.0</td>
<td>0.15b/0.15b</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>1.0/22.0</td>
<td>76</td>
<td>1.0/22.0</td>
<td>0.1/0.15b</td>
<td>113</td>
<td>22.0/22.0</td>
<td>0.15b/0.15b</td>
<td></td>
</tr>
</tbody>
</table>
Table 2 – Class I SLA-1 Effect on Virus and Serum Neutralizing Antibody (SNA) Level

<table>
<thead>
<tr>
<th>Allele</th>
<th>N#</th>
<th>Virus$^1$</th>
<th>SNA$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>rh03</td>
<td>3</td>
<td>-98.32 ± 64.62</td>
<td>13.45 ± 26.24</td>
</tr>
<tr>
<td>01XX</td>
<td>50</td>
<td>37.93 ± 28.64</td>
<td>10.43 ± 11.81</td>
</tr>
<tr>
<td>04XX</td>
<td>43</td>
<td>52.42 ± 29.01</td>
<td>0.31 ± 12.09</td>
</tr>
<tr>
<td>07XX</td>
<td>5</td>
<td>-374.38$^A$ ± 53.25</td>
<td>-34.25 ± 21.71</td>
</tr>
<tr>
<td>08XX</td>
<td>43</td>
<td>44.90 ± 27.02</td>
<td>-11.26 ± 11.12</td>
</tr>
<tr>
<td>11XX</td>
<td>1</td>
<td>113.19 ± 107.39</td>
<td>-32.14 ± 43.61</td>
</tr>
<tr>
<td>06XX, 08XX</td>
<td>1</td>
<td>25.90 ± 108.65</td>
<td>20.15 ± 44.17</td>
</tr>
<tr>
<td>09XX, 15XX</td>
<td>11</td>
<td>21.34 ± 39.96</td>
<td>9.30 ± 16.75</td>
</tr>
<tr>
<td>Blank</td>
<td>32</td>
<td>62.20 ± 30.29</td>
<td>-13.86 ± 12.57</td>
</tr>
<tr>
<td>Blank, 01XX</td>
<td>1</td>
<td>114.80 ± 107.39</td>
<td>37.86 ± 43.61</td>
</tr>
</tbody>
</table>

$^A$Total number of times allele was observed in analysis of virus and SNA

$^1$Allele effect on virus level ± standard error; difference from mean (μ=168.9 log of parts/ml)

$^2$Allele effect on SNA level ± standard error; difference from mean (μ=88.9 log base 2 of dilution)

$^A$Significant effect p<0.05
### Table 3 – Class I SLA-1 Effect on IL8, IL1b, and IFNγ Level

<table>
<thead>
<tr>
<th>Allele</th>
<th>N#</th>
<th>IL8</th>
<th>IL1b</th>
<th>IFNγ</th>
</tr>
</thead>
<tbody>
<tr>
<td>rh03</td>
<td>1</td>
<td>-16.29 ± 32.91</td>
<td>-31.78 ± 52.67</td>
<td>-1.78 ± 4.87</td>
</tr>
<tr>
<td>01XX</td>
<td>22</td>
<td>16.84 ± 11.99</td>
<td><strong>-36.26B ± 19.20</strong></td>
<td>-0.14 ± 1.78</td>
</tr>
<tr>
<td>04XX</td>
<td>20</td>
<td><strong>22.62B ± 11.55</strong></td>
<td>2.37 ± 18.49</td>
<td>0.09 ± 1.72</td>
</tr>
<tr>
<td>07XX</td>
<td>2</td>
<td><strong>-83.41A ± 23.89</strong></td>
<td>37.89 ± 38.23</td>
<td>4.66 ± 3.53</td>
</tr>
<tr>
<td>08XX</td>
<td>23</td>
<td><strong>23.26A ± 9.72</strong></td>
<td>-20.65 ± 15.56</td>
<td>0.74 ± 1.44</td>
</tr>
<tr>
<td>11XX</td>
<td>1</td>
<td>34.82 ± 32.91</td>
<td>-31.78 ± 52.67</td>
<td>-1.78 ± 4.87</td>
</tr>
<tr>
<td>09XX, 15XX</td>
<td>3</td>
<td>-9.58 ± 20.69</td>
<td><strong>56.86B ± 33.12</strong></td>
<td>-2.08 ± 3.08</td>
</tr>
<tr>
<td>Blank</td>
<td>14</td>
<td>11.74 ± 11.58</td>
<td>23.35 ± 18.53</td>
<td>0.29 ± 1.88</td>
</tr>
</tbody>
</table>

*NTotal number of times allele was observed in analysis of IL8, IL1b, and IFNγ

1Allele effect on IL8 level ± standard error; difference from mean (µ=70.5 log of pg/ml)

2Allele effect on IL1b level ± standard error; difference from mean (µ=84.1 log of pg/ml)

3Allele effect on IFNγ level ± standard error; difference from mean (µ=53.3 log of pg/ml)

ASignificant effect p<0.05

BSuggestive effect p<0.10

### Table 4 – Class I SLA-2 Effect on IL8, IL1b, and IFNγ Level

<table>
<thead>
<tr>
<th>Allele</th>
<th>N#</th>
<th>IL8</th>
<th>IL1b</th>
<th>IFNγ</th>
</tr>
</thead>
<tbody>
<tr>
<td>01XX</td>
<td>27</td>
<td>-8.37 ± 11.47</td>
<td>-22.66 ± 16.62</td>
<td>-0.23 ± 1.33</td>
</tr>
<tr>
<td>04XX</td>
<td>18</td>
<td>0.29 ± 14.17</td>
<td>-5.34 ± 20.54</td>
<td>-2.16 ± 1.64</td>
</tr>
<tr>
<td>05XX</td>
<td>9</td>
<td>-6.21 ± 16.41</td>
<td>-0.13 ± 23.79</td>
<td>0.59 ± 1.90</td>
</tr>
<tr>
<td>07XX</td>
<td>1</td>
<td>21.44 ± 38.44</td>
<td>-45.88 ± 55.71</td>
<td>-3.50 ± 4.45</td>
</tr>
<tr>
<td>08XX</td>
<td>2</td>
<td>-1.18 ± 28.72</td>
<td><strong>72.99B ± 41.63</strong></td>
<td>7.76A ± 3.34</td>
</tr>
<tr>
<td>10XX</td>
<td>11</td>
<td>-7.25 ± 15.48</td>
<td>20.88 ± 22.43</td>
<td>-1.87 ± 1.85</td>
</tr>
<tr>
<td>12XX</td>
<td>18</td>
<td>1.28 ± 11.01</td>
<td>-19.87 ± 15.96</td>
<td>-0.60 ± 1.28</td>
</tr>
</tbody>
</table>

*NTotal number of times allele was observed in analysis of IL8, IL1b, and IFNγ

1Allele effect on IL8 level ± standard error; difference from mean (µ=70.5 log of pg/ml)

2Allele effect on IL1b level ± standard error; difference from mean (µ=84.1 log of pg/ml)

3Allele effect on IFNγ level ± standard error; difference from mean (µ=53.3 log of pg/ml)

ASignificant effect p<0.05

BSuggestive Effect p<0.10
Table 5 – Effect of SLA Class II Haplotype on Serum Neutralizing Antibody (SNA)

<table>
<thead>
<tr>
<th>Day</th>
<th>Ln#</th>
<th>28²</th>
<th>42³</th>
<th>56⁴</th>
<th>70⁵</th>
<th>84⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31</td>
<td>-0.026 ± 0.088</td>
<td>-0.343 ± 0.328</td>
<td>-0.524 ± 0.264</td>
<td>-0.399 ± 0.341</td>
<td><strong>-0.732B ± 0.301</strong></td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>0.124 ± 0.116</td>
<td>-0.289 ± 0.432</td>
<td>-0.171 ± 0.359</td>
<td>0.042 ± 0.474</td>
<td>-0.736 ± 0.410</td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>0.106 ± 0.110</td>
<td>0.089 ± 0.411</td>
<td>-0.276 ± 0.339</td>
<td>-0.121 ± 0.436</td>
<td>0.118 ± 0.382</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
<td>-0.050 ± 0.186</td>
<td>-0.432 ± 0.695</td>
<td>0.064 ± 0.558</td>
<td>-0.321 ± 0.708</td>
<td>-0.069 ± 0.614</td>
</tr>
<tr>
<td>13</td>
<td>4</td>
<td>-0.027 ± 0.193</td>
<td>0.303 ± 0.721</td>
<td>-0.519 ± 0.587</td>
<td>0.797 ± 0.745</td>
<td>-0.161 ± 0.645</td>
</tr>
<tr>
<td>15</td>
<td>31</td>
<td>0.039 ± 0.085</td>
<td>-0.094 ± 0.319</td>
<td>-0.533 ± 0.258</td>
<td>-0.400 ± 0.329</td>
<td><strong>-1.000A ± 0.290</strong></td>
</tr>
<tr>
<td>19</td>
<td>1</td>
<td>-0.092 ± 0.359</td>
<td>-0.012 ± 1.342</td>
<td>0.031 ± 1.078</td>
<td>-1.942 ± 1.368</td>
<td>-0.416 ± 1.185</td>
</tr>
<tr>
<td>22</td>
<td>1</td>
<td>-0.092 ± 0.359</td>
<td>0.988 ± 1.342</td>
<td>1.031 ± 1.078</td>
<td>1.058 ± 1.368</td>
<td>0.584 ± 1.185</td>
</tr>
<tr>
<td>23</td>
<td>24</td>
<td>0.120 ± 0.092</td>
<td>-0.438 ± 0.343</td>
<td>-0.663 ± 0.280</td>
<td>-0.440 ± 0.362</td>
<td>-0.568 ± 0.314</td>
</tr>
<tr>
<td>24</td>
<td>6</td>
<td>-0.039 ± 0.166</td>
<td>0.440 ± 0.621</td>
<td>0.413 ± 0.556</td>
<td>0.809 ± 0.707</td>
<td>0.982 ± 0.614</td>
</tr>
<tr>
<td>25</td>
<td>3</td>
<td>-0.053 ± 0.213</td>
<td>-0.692 ± 0.794</td>
<td>0.909 ± 0.637</td>
<td>-0.076 ± 0.808</td>
<td><strong>1.763B ± 0.700</strong></td>
</tr>
<tr>
<td>26</td>
<td>22</td>
<td>-0.010 ± 0.104</td>
<td>0.480 ± 0.389</td>
<td>0.237 ± 0.315</td>
<td>0.992 ± 0.407</td>
<td>0.537 ± 0.365</td>
</tr>
</tbody>
</table>

²Total number of times haplotype was observed in analysis of SNA
³Number of days post inoculation
²SLA haplotype effect on SNA level ± standard error; difference from mean (µ=0.08 log base 2 of dilution)
³SLA haplotype effect on SNA level ± standard error; difference from mean (µ=1.72 log base 2 of dilution)
⁴SLA haplotype effect on SNA level ± standard error; difference from mean (µ=2.70 log base 2 of dilution)
⁵SLA haplotype effect on SNA level ± standard error; difference from mean (µ=2.03 log base 2 of dilution)
⁶SLA haplotype effect on SNA level ± standard error; difference from mean (µ=1.66 log base 2 of dilution)
A Significant Effect p<0.05
B Suggestive Effect p<0.10
Table 6 – Effect of SLA Class II Haplotype within Day on IFNγ Level

<table>
<thead>
<tr>
<th>Lr</th>
<th>N#</th>
<th>7</th>
<th>14</th>
<th>28</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.029 ± 0.064</td>
<td>0.000 ± 0.000</td>
<td>0.078 ± 0.157</td>
<td>-0.044 ± 0.112</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>0.199 ± 0.094</td>
<td>0.000 ± 0.000</td>
<td>-0.144 ± 0.231</td>
<td>-0.233 ± 0.165</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>-0.065 ± 0.079</td>
<td>0.000 ± 0.000</td>
<td>-0.098 ± 0.194</td>
<td><strong>-0.441A ± 0.138</strong></td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>-0.023 ± 0.140</td>
<td>0.000 ± 0.000</td>
<td>-0.158 ± 0.344</td>
<td>-0.258 ± 0.245</td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>-0.004 ± 0.141</td>
<td>0.000 ± 0.000</td>
<td>0.666 ± 0.345</td>
<td>0.424 ± 0.246</td>
</tr>
<tr>
<td>15</td>
<td>16</td>
<td>-0.027 ± 0.055</td>
<td><strong>7.29e-16A ± 1.49e-16</strong></td>
<td>0.012 ± 0.135</td>
<td>-0.174 ± 0.096</td>
</tr>
<tr>
<td>19</td>
<td>1</td>
<td>0.001 ± 0.195</td>
<td>0.000 ± 0.000</td>
<td>-0.072 ± 0.477</td>
<td>-0.020 ± 0.340</td>
</tr>
<tr>
<td>23</td>
<td>10</td>
<td>-0.009 ± 0.070</td>
<td>0.000 ± 0.000</td>
<td>-0.090 ± 0.173</td>
<td>-0.173 ± 0.123</td>
</tr>
<tr>
<td>25</td>
<td>2</td>
<td>-0.046 ± 0.139</td>
<td>0.000 ± 0.000</td>
<td>-0.160 ± 0.340</td>
<td><strong>1.150A ± 0.242</strong></td>
</tr>
<tr>
<td>26</td>
<td>12</td>
<td>-0.055 ± 0.074</td>
<td>0.000 ± 0.000</td>
<td>-0.036 ± 0.181</td>
<td>-0.232 ± 0.129</td>
</tr>
</tbody>
</table>

#Total number of times haplotype was observed in analysis of IFNγ

1Number of days post inoculation

2SLA haplotype effect on IFNγ level ± standard error; difference from mean
   (µ=1.52 log of pg/ml)

3SLA haplotype effect on IFNγ level ± standard error; difference from mean
   (µ=1.51 log of pg/ml)

4SLA haplotype effect on IFNγ level ± standard error; difference from mean
   (µ=1.56 log of pg/ml)

5SLA haplotype effect on IFNγ level ± standard error; difference from mean
   (µ=1.58 log of pg/ml)

A Significant effect p<0.05
Table 7 – Effect of SLA Class I Haplotype within Day on IL1b Level

<table>
<thead>
<tr>
<th>Lr</th>
<th>N #</th>
<th>7²</th>
<th>14³</th>
<th>28⁴</th>
<th>42⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>-0.653 ± 0.256</td>
<td>-0.754 ± 0.318</td>
<td><strong>-0.372 B ± 0.139</strong></td>
<td>-0.237 ± 0.106</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>-0.182 ± 0.304</td>
<td>-0.176 ± 0.378</td>
<td>-0.330 ± 0.165</td>
<td>-0.127 ± 0.125</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>1.318 ± 0.587</td>
<td>1.104 ± 0.730</td>
<td><strong>1.175 A ± 0.318</strong></td>
<td><strong>0.720 A ± 0.243</strong></td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>-0.550 ± 0.469</td>
<td>-0.661 ± 0.584</td>
<td>-0.230 ± 0.254</td>
<td>-0.229 ± 0.194</td>
</tr>
<tr>
<td>21</td>
<td>1</td>
<td>-0.570 ± 0.806</td>
<td>-0.692 ± 1.002</td>
<td>-0.188 ± 0.437</td>
<td>-0.119 ± 0.333</td>
</tr>
<tr>
<td>22</td>
<td>18</td>
<td>-0.338 ± 0.233</td>
<td>-0.389 ± 0.290</td>
<td>-0.092 ± 0.127</td>
<td>-0.147 ± 0.096</td>
</tr>
<tr>
<td>25</td>
<td>1</td>
<td>-0.570 ± 0.806</td>
<td>-0.692 ± 1.002</td>
<td>-0.188 ± 0.437</td>
<td>-0.119 ± 0.333</td>
</tr>
<tr>
<td>27</td>
<td>1</td>
<td>-0.835 ± 0.836</td>
<td>-0.001 ± 1.040</td>
<td>-0.644 ± 0.453</td>
<td>-0.270 ± 0.345</td>
</tr>
<tr>
<td>28</td>
<td>3</td>
<td>1.129 ± 0.495</td>
<td>1.011 ± 0.615</td>
<td>0.599 ± 0.268</td>
<td>0.260 ± 0.204</td>
</tr>
<tr>
<td>30</td>
<td>2</td>
<td>1.026 ± 0.581</td>
<td>0.693 ± 0.722</td>
<td>-0.209 ± 0.315</td>
<td>-0.174 ± 0.240</td>
</tr>
<tr>
<td>31</td>
<td>2</td>
<td>1.051 ± 0.585</td>
<td>0.952 ± 0.728</td>
<td><strong>0.863 B ± 0.317</strong></td>
<td><strong>0.738 A ± 0.242</strong></td>
</tr>
<tr>
<td>34</td>
<td>1</td>
<td>-0.885 ± 0.812</td>
<td>-0.407 ± 1.010</td>
<td>-0.468 ± 0.440</td>
<td>-0.210 ± 0.335</td>
</tr>
<tr>
<td>39</td>
<td>11</td>
<td>0.058 ± 0.355</td>
<td>0.010 ± 0.442</td>
<td>0.084 ± 0.192</td>
<td>-0.086 ± 0.147</td>
</tr>
</tbody>
</table>

#Total number of times haplotype was observed in analysis of IL1b

1 Number of days post inoculation

2 SLA haplotype effect on IL1b level ± standard error; difference from mean (\(\mu=2.53\) log of pg/ml)

3 SLA haplotype effect on IL1b level ± standard error; difference from mean (\(\mu=2.58\) log of pg/ml)

4 SLA haplotype effect on IL1b level ± standard error; difference from mean (\(\mu=2.31\) log of pg/ml)

5 SLA haplotype effect on IL1b level ± standard error; difference from mean (\(\mu=2.25\) log of pg/ml)

A Significant effect p<0.05

B Suggestive effect p<0.10
Table 8 – Effect of SLA Class II Haplotype within Day on Virus Level

<table>
<thead>
<tr>
<th>Lr</th>
<th>N#</th>
<th>7²</th>
<th>14³</th>
<th>28⁴</th>
<th>42⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>31</td>
<td>-0.674 ± 0.471</td>
<td>-0.744 ± 0.585</td>
<td>0.473 ± 0.727</td>
<td>-0.009 ± 0.800</td>
</tr>
<tr>
<td>0.2</td>
<td>13</td>
<td>-0.583 ± 0.621</td>
<td>0.301 ± 0.771</td>
<td>-0.014 ± 0.959</td>
<td>0.739 ± 1.055</td>
</tr>
<tr>
<td>0.4</td>
<td>32</td>
<td>-0.110 ± 0.591</td>
<td>0.552 ± 0.734</td>
<td>1.182 ± 0.912</td>
<td>0.307 ± 1.004</td>
</tr>
<tr>
<td>0.12</td>
<td>4</td>
<td>-1.840 ± 0.998</td>
<td>-5.428A ± 1.240</td>
<td>0.705 ± 1.541</td>
<td>2.024 ± 1.697</td>
</tr>
<tr>
<td>0.13</td>
<td>4</td>
<td>1.858 ± 1.036</td>
<td>2.199 ± 1.287</td>
<td>1.149 ± 1.600</td>
<td>-0.590 ± 1.761</td>
</tr>
<tr>
<td>0.15b</td>
<td>31</td>
<td>0.404 ± 0.458</td>
<td>0.978 ± 0.570</td>
<td>-0.333 ± 0.708</td>
<td>-0.160 ± 0.779</td>
</tr>
<tr>
<td>0.19</td>
<td>1</td>
<td>1.678 ± 1.927</td>
<td>2.210 ± 2.394</td>
<td>-1.919 ± 2.976</td>
<td>-2.037 ± 3.276</td>
</tr>
<tr>
<td>0.22</td>
<td>1</td>
<td>0.076 ± 1.927</td>
<td>-2.640 ± 2.394</td>
<td>-1.338 ± 2.976</td>
<td>-2.037 ± 3.276</td>
</tr>
<tr>
<td>0.23</td>
<td>24</td>
<td>0.555 ± 0.493</td>
<td>0.344 ± 0.613</td>
<td>0.921 ± 0.762</td>
<td>1.529 ± 0.838</td>
</tr>
<tr>
<td>0.24</td>
<td>6</td>
<td>1.096 ± 0.892</td>
<td>1.680 ± 1.109</td>
<td>-0.687 ± 1.378</td>
<td>0.414 ± 1.517</td>
</tr>
<tr>
<td>0.25</td>
<td>3</td>
<td>-3.627A ± 1.140</td>
<td>-0.765 ± 1.416</td>
<td>-0.433 ± 1.760</td>
<td>-0.758 ± 1.937</td>
</tr>
<tr>
<td>0.26</td>
<td>22</td>
<td>1.165 ± 0.558</td>
<td>1.313 ± 0.693</td>
<td>0.296 ± 0.862</td>
<td>0.276 ± 0.949</td>
</tr>
</tbody>
</table>

# Total number of times haplotype was observed in analysis of virus titer
1 Number of days post inoculation
2 SLA haplotype effect on virus level ± standard error; difference from mean (μ=6.89 log of parts/ml)
3 SLA haplotype effect on virus level ± standard error; difference from mean (μ=6.81 log of parts/ml)
4 SLA haplotype effect on virus level ± standard error; difference from mean (μ=4.72 log of parts/ml)
5 SLA haplotype effect on virus level ± standard error; difference from mean (μ=1.02 log of parts/ml)
A Significant Effect p<0.05
Chapter 4: Effect of interleukin 1b, interleukin 8 and interferon gamma on PRRS virus persistence

Joan K. Lunney¹, Eric R Fritz²#, James M Reecy², Daniel Kuhar¹, Elizabeth Prucnal¹, Ramon Molina³, Jane Christopher-Hennings⁴, Jeffrey Zimmerman³, Raymond R R Rowland⁵

¹APDL, BARC, USDA, Beltsville, MD
²Department of Animal Science, Iowa State University, Ames, IA 50011-3150,
³Iowa State University, Ames IA
⁴South Dakota State University, Brookings SD
⁵Kansas State University, Manhattan, KS

This journal paper was funded by contributions from the National Pork Board, PRRS CAP1, USDA Agricultural Research Service and USDA Cooperative State Research, Education, and Extension Service.

#ERF contributions include development and implementation of the database as well as statistical analysis.
Abstract

Infection with Porcine Reproductive and Respiratory Syndrome virus (PRRSV) elicits a weak immune response that is weakly protective and results in persistent infection in a subset of pigs. We investigated the intensity and timing of the early cytokine responses to PRRSV infection to determine their utility as a predictor of final PRRSV burden and persistence. As part of the “Big Pig” project we tested gene and protein expression in immune tissues collected from pigs at kill and serum samples collected biweekly for up to 202 days post-PRRSV infection (dpi). We compared pigs that apparently cleared the viral infection from serum and tissues, the non-persistent (NP) pigs, to persistent (P) pigs which had virus in serum and/or tissues at kill. Gene expression studies in tracheobronchial LN (TBLN) samples collected at kill demonstrated that there is up regulation of expression of interferon-gamma (IFNγ) associated T helper 1 (Th1) markers from 14 to 84 dpi, of T-regulatory, IL10, and but no upregulation of innate markers (IFNA, IL1b, IL8). At later time points (>112 dpi, the time chosen for persistence analyses) these genes are no longer differentially expressed and thus are uninformative for persistence studies. We next asked whether immune cytokine (IL-1b, IL-8, IFNγ) levels early after infection determine why certain pigs have persistent PRRSV infections. Statistical analyses indicated that innate cytokines IL-1b and IL-8 levels were up-regulated early after infection, with a significant difference between NP and P pig levels at 14 dpi for IL-8; serum IFNγ levels peaked at 28 dpi. When analyzed together, variation in all 3 of the cytokines tested (IL-8, IL-1b and IFNγ) had a significant effect on virus level, accounting for ~84% of the variation observed, however, it is not known if this is due to a direct
or indirect effect of these cytokines. These results indicate that while each cytokine individually has minor effects on persistency, the combination of cytokines could be used to potentially determine which pigs will have persistent virus infections.

**Introduction**

Porcine Reproductive and Respiratory Syndrome (PRRS), when present in a herd is manifested by increased rates of abortions, stillbirths, mummifications, and chronic respiratory problems. Economically, PRRS costs the US pork industry $560 million annually (Neumann et al. 2005). A major obstacle for the control and prevention of PRRSV infection in pigs is the capacity of the virus to become persistent within a production system. In 1997, Wills et al. isolated PRRSV from pigs at 157 days post infection (dpi) (Wills et al. 1997). Allende et al. (2000) proposed that this persistence may be due to continuous viral replication, as opposed to true steady-state persistent infection. Despite being seronegative persistently infected pigs still harbor PRRSV and have been proven to be a source of virus for susceptible animals (Wills et al. 2003). Indeed, RT-PCR analysis revealed that viral RNA was present until 202 days post infection (dpi) in the current “BigPig” experimental samples (Molina et al. 2008a,b). Semen persistence of PRRSV adds to this problem (Christopher-Hennings et al. 2001). Congenital infection also results in pigs that are persistently PRRSV positive and transmit PRRSV (Bierk et al. 2001; Rowland et al. 2003). Only by 260 dpi did pigs became seronegative and not transmit virus to
sentinel pigs, at 300 days pigs showed no evidence of virus in blood and tissues (Rowland et al. 2003). Indeed, these time points for possible clearance of PRRS virus are well beyond the life time of a pig. They highlight the impact of this virus on the every stage of pork production; thus, the heavy economic losses (Neumann et al. 2005).

Horter et al. (2002; 2003) found that infectious PRRS virus is present in most pigs the first 105 dpi and suggested that RT-PCR assays of oropharyngeal scrapings was the most effective combination of assay and sample for detecting PRRSV carriers. They noted that antibody response levels did not distinguish PRRSV carrier from non-carrier animals. Serum virus neutralizing antibodies (SNA) are important in PRRSV control during acute infection (Lopez and Osorio, 2004; Osorio et al. 2002), but polyclonal B cell activation also occurs (Lamontagne et al. 2001; Lemke et al. 2004; Plagemann et al. 2005). Moreover, Batista et al. (2004) confirmed that none of an extensive set of parameters [serum ELISA antibody, SNA, interferon-γ (IFNγ) levels using ELISpot and flow cytometry, proliferative responses of CD4+ and CD8+ T lymphocytes], either alone or in combination, is definitively associated with (or predictive of) viral clearance and sterilizing immunity. PRRSV persists in the serum, despite SNA, and in the lymphoid tissue, even after IFNγ levels dropped, thus indicating that some other immune factor is associated with viral clearance and sterilizing immunity (Batista et al. 2004). In field studies Lowe et al. (2005) indicated the importance of cell-mediated immunity (IFNγ levels using ELISpot) in preventing
piglet losses from sows, although genetic factors also were indicated. CD8+ T cell depletion had no effect on PRRSV resistance (Lohse et al. 2002).

Thus in this study, we asked whether immune factors, and particularly immune cytokines [Interleukin-1β (IL-1β), IL-8, IFNγ] help determine why certain pigs have long-term persistent PRRSV infections. The availability of samples collected during the “BigPig” study provided sera and tissues collected biweekly for up to 202 days of PRRSV infection (Molina et al. 2008a,b).

Materials and Methods

Animal care, handling, and sampling

As previously described by Molina et al. (Molina et al. 2008a,b), 109 Large White/Landrace cross bred pigs were infected with PRRSV and followed for 202 days. Specifically, on day 0, pigs (n = 109) were intramuscularly inoculated with 1 ml of PRRSV (VR-2332 1 X 10^4.0 TCID_{50}) diluted in minimum essential medium. Control pigs were inoculated with PRRSV-negative MEM by the same method. Blood samples were collected from the pigs on day 0 before inoculation, at 7 and 14 days post-inoculation (dpi), and every 14 days after up to 202 dpi.

Virus strain and propagation

The North American prototype PRRSV, American Type Culture Collection [ATCC] VR-2332 was used in this study as previously described (Molina et al. 2008a,b). Plaque-cloned virus was propagated by inoculation of a 21-day-old pig, collection of serum on 7 dpi; followed by its inoculation onto 24-hr-old confluent
MARC-145 cells. After freeze-thaw cell lysates were harvested and stored at -80°C (Molina et al. 2008a,b).

**Viral RNA quantitation using reverse-transcriptase polymerase chain reaction (RT-PCR)**

Quantitative, real-time RT-PCR (qPCR) was used to determine viral levels as previously described (Wasilik et al. 2005) using a commercially available kit (Tetracore Inc., Rockville, MD, USA). Quantification of sample viral levels was calculated and expressed as number of RNA copies per ml for fluid samples or number of RNA copies per gram for solid tissue samples. Virus levels were quantitated for inguinal lymph node (IngLN), submaxillary LN (SubLN), and tonsils. More tissues were collected for gene expression work including lung and tracheobronchial LN (TBLN) (Molina et al. 2008a,b).

**Tissue gene expression analyses**

Real-time PCR for immune marker gene detection was performed as previously described (Royaee et al. 2004; Dawson et al. 2005). The BigPig protocol set up a planned kill of 5-10 infected pigs at each dpi (Molina et al. 2008a,b). Gene expression data was determined for RNA isolated from TBLN samples from individual pigs and compared to a randomly selected set of control BigPig TBLN samples collected over the course of the study. Each cDNA sample was analyzed for expression of 23 immune related genes and one housekeeping gene (RPL32). Genes evaluated are involved in both innate and acquired
immunity. Relative quantification of target gene expression was evaluated using cycle threshold (Ct) values and recorded with the Applied Biosystems PRISM 7700 Sequence Detector System. Each individual's data was calculated, then averaged for the dpi and the group's results were compared to the values for averages of the control pigs using the JMP statistical package (Cary, NC) as previously published (Royaee et al. 2004; Dawson et al. 2005). Least squares means analyses were used to statistically evaluate group means for immune gene analyses. For individual comparisons each gene Ct value was compared to the control average for that gene.

_Cytokine protein analysis_

The cytokines IL-1β, IL-8, and IFNγ were quantified in sequential serum samples collected at 14-day intervals from 91 of the 109 PRRSV-inoculated animals. Levels of IL-1β and IL-8 were evaluated in samples collected through 42 dpi and IFNγ in samples collected through 84 dpi. Serum samples were assayed with commercial ELISA kits (IL-1β and IL-8: R&D Systems, Minneapolis, MN, USA 2; IFNγ: Biosource™ Invitrogen, Camarillo, CA) following protocols provided by the manufacturers. The substrate, SureBlue™ (KPL, Inc., Gaithersburg, MD), was added and plates were read at 650 nm until the highest standard reached an OD of ~2.0 with no acid added. For all ELISA data, the OD of the medium controls was averaged and subtracted from the OD of each sample and then the lowest readable concentration was determined from the standard curve. If the OD was below the lowest level, values were assigned by dividing the lowest
readable standard concentration by 2 and then multiplying that number by the
dilution factor to assign minimal detectable levels.

Statistical analysis

Data was analyzed using the PROC-GLM and least squares-means
methods of SAS (SAS Institute Inc., Cary, NC, USA). Initially the data were
analyzed with the following statistical to determine if IL-8, IL-1b, and IFNγ had an
effect on virus and serum neutralizing antibody (SNA) level.

\[
y = \mu + a + b + c + d + a \times d + c \times d
\]

(Full Model)

where \( y \) is virus or SNA level, \( \mu \) is the general mean, \( a \) is the effect of IL-8, \( b \) is
the effect of IL-1b, \( c \) is the effect of IFNγ, \( d \) is the effect of day, \( a \times d \) is the effect
of the interaction of day and IL-8, and \( c \times d \) is the effect of the interaction of day
and IFNγ.

Interest in persistency (a pig was classified as persistent if at kill virus was
not cleared from serum or tissues) of viral infection led to an individual analysis of
whether or not each of the following, virus level, SNA level, IL-8 level, IL-1b, level
or IFNγ level, had an effect on whether or not a pig was classified as a persistent
carrier of the PRRS virus or not.

\[
y = \mu + a + b + a \times b
\]

(Single Trait Model)

where \( y \) is virus level, SNA, IL-8, IL-1b, or IFNγ level, \( \mu \) is some mean, \( a \) is the
effect of the persistence classification (persistent or non-persistent), \( b \) is the
effect of day, and \( a \times b \) is the effect of the interaction of day and the persistence
classification.
**Database information**

The PRRS Host Genome Consortium database contained the data used in this study and has been described by Fritz et al. (2009).

**Results**

**Assignment of Persistence status and viral clearance**

The PRRSV persistence status was determined based on viral RNA qPCR analyses of serum and tissues, tonsils, inguinal lymph node (IngLN) and submaxillary LN (SubLN) from the Big Pig Project (Molina et al. 2008a,b). Non-Persistent Pigs (NP) are defined as those pigs which cleared infection, i.e., were negative for evidence of PRRSV by viral RNA qPCR analyses of serum at 28 dpi or later and tissues at time of euthanasia (112 dpi to 202 dpi.). Persistent Pigs (P) are those with proven persistent infections, i.e., positive for evidence of PRRSV by viral RNA qPCR analyses of serum and/or lymphoid tissues and/or tonsils at time of euthanasia (112 dpi to 202 dpi). The P pigs were serum virus qPCR positive at least until 28 dpi (average for 68 dpi); several were serum virus qPCR positive until 98-126 dpi (Table 1).

Analyses of serum viral levels of NP and P pigs illustrated an important difference between the P versus NP groups of pigs (Figure 1). There was a statistically significant (P<0.05) difference in serum viral levels as early as 28 dpi which became more significant at 42 dpi (P<0.01) when the NP pigs had completely cleared serum virus.
Gene Expression for immune markers in TBLN

Our original goal for the BigPig persistence studies was to determine whether gene expression for immune markers in local tissues could identify factors associated with long term persistence. To this end RNA prepared from infected pig TBLN collected during the Big Pig studies were tested for expression of a panel of immune markers (Figure 2A). Samples from each dpi were tested, Ct values averaged and the gene expression data for each dpi averaged and compared to the average Ct for control tissues. The results showed that there was up regulation of expression of IFNγ associated T helper 1 (Th1) markers, with the highest up regulation from 42 to 84 dpi. Importantly, there was no up regulation of most innate markers (IFNA, IL1B, IL6, IL8) a key factor in the poor immune response to PRRSV infection. Moreover there was up-regulation of the T-regulatory (T-reg) marker, IL10 as early as D14, and chemokine and apoptosis markers at 42-56 dpi. By 112 dpi most immune markers were lower than the averaged control tissues. This indicated that we would be unlikely to get informative comparisons for our studies of persistence targeting 112-202 dpi based on gene expression differences of respiratory tissues. The BigPig protocol set up a planned kill of 10 pigs at each dpi. This data was evaluated as outlined in Table 1 and resulted in a low number of designated NP or P pigs at each dpi. Thus individual pig data gene expression data (relative to a random set of controls killed on the same dpi) is shown in Fig. 2B with very low differential gene expression for most genes. Thus when the TBLN immune gene expression
(relative to controls) of NP and P pigs were compared there were no apparent
differences in gene expression when assayed at day of kill.

*Serum Immune Cytokine Protein Expression as a predictor of PRRSV persistence.*

The failure of the gene expression approach made us reevaluate our
assumptions. We then hypothesized that early immune events, i.e., the intensity
and timing of the early cytokine responses to PRRSV infection, might be a
predictor of final PRRSV burden. Therefore going back to the samples banked
through the BigPig project, we tested sera for cytokine protein induction in
response to infection with PRRSV. Table 2 shows the numbers of pigs for which
these analyses were performed. Overall samples from 91 control and infected
 pigs were analyzed for serum cytokine levels. Of these 44 infected pigs killed at
112 dpi or later were analyzed for the more detailed statistical comparisons of
levels of serum cytokines expressed by P or NP pigs and their role in
 persistence. Data from the 44 PRRSV-infected pigs were analyzed for effects of
serum cytokine levels on PRRSV levels using the PROC-GLM function of SAS
with full model. The model was significant (p<0.0001) with an $R^2=0.84$ (Table 2).
The main effects of IL-8, IL-1b, and day were significant (p<0.01), while the main
effect of IFNγ was not significant (p>0.10). The interaction of day X IL-8 was
significant (p<0.05), while the interaction of day X IFNγ was suggestive (p<0.10).

Statistical analyses indicated that innate cytokines (IL-1b, IL-8) were
increased earliest, followed by IFNγ. The infected pigs exhibited high IL-1b to 14
dpi when the persistently infected pigs showed a higher concentration of IL-1b than P animals. When IL-8 was examined at dpi 14, NP pigs had higher concentrations of IL-8 than P pigs. There appeared to be no changes in serum IL-10 associated with infection (data not shown). Overall, pigs with non-persistent PRRSV infections appeared to have earlier and higher IL-8 levels than the pigs with persistent infections.

Using the full model, data from individuals labeled as persistent were run through the PROC-GLM function of SAS. This model was significant ($p<0.0001$) with an $R^2=0.75$ (Table 1). The main effect of day was significant ($p<0.01$), while the main effect of IL-1b was only suggestive ($p<0.10$) and the main effects of IL-8 and IFNγ were not significant. The interaction of day X IL-8 was significant ($p<0.05$), while the interaction of day X IFNγ was not significant ($p>0.10$).

Data from individual pigs labeled as non-persistent were analyzed with the full model, which was significant ($p<0.0001$) with an $R^2=0.89$ (Table 2). The main effect of day was significant ($p<0.01$), while the main effect of IL-1b was suggestive ($p<0.10$). In contrast, the main effects of IL-8 and IFNγ were not significant ($p>0.10$). The interaction of day X IL-8 was not significant ($p>0.10$) and the interaction of day X IFNγ was suggestive ($p<0.10$).

Effect of persistence

Data from the 91 PRRSV-infected pigs were run through the PROC-GLM function of SAS using the single trait model. The model was run using virus, IL-8, IL-1b, and IFNγ level. The single trait model was significant for virus. The main
effects of persistence and day as well as the interaction of persistence X day were all significant (p<0.01; Table 1). The virus level significantly decreases on dpi 28 and then again on dpi 42. There is also a significant difference between persistent and non-persistent pigs on dpi 28 and 42.

The single trait model was significant for IL-8. The main effect of day was significant (p<0.01; Figure 3A), while the main effect of persistence and the interaction of persistence X day were not significant (p>0.10; Figure 3B). Interleukin-8 levels peaked on dpi 14 and then significantly decreased on 28 dpi continuing to 42 dpi. The single trait model was also significant for IL-1β. The main effect of day was significant (p<0.01; Figure 2C), while the main effect of persistence and the interaction of persistence X day were not significant (p>0.10; Figure 2D). Interleukin-1β levels were similar for 7 and 14 dpi which were significantly higher than both 28 and 42 dpi. The single trait model was not significant for IFNγ (p>0.10). The main effects of day (Figure 2E), persistence and the interaction of persistence X day (Figure 2F) were all not significant except for the NP pigs (p>0.10). Interferon gamma levels were moderately increased at 28 dpi and statistically different only in the NP pigs at 28dpi from the 14 dpi values; no differences were seen at 42 dpi.

Discussion

Numerous studies have affirmed PRRSV persistence in tissues for extended time periods (Wills et al. 1997; 2003; Allende et al. 2000; Rowland et al. 2003; Batista et al., 2004; ). Our data has affirmed that persistence out to 147
days (using viral RT-PCR as the measure; Molina et al. 2008a,b). The underlying mechanism supporting, or preventing, PRRSV persistence in tissues has not yet been identified. In this manuscript we have presented data that supports the hypothesis that cytokine levels early after PRRSV infection may help determine whether the virus will persist in blood and tissues. Mucosal respiratory tissue (TBLN) gene expression data at day of kill did not reveal any differential reactivity of RNA from P versus NP pigs.

Sera, collected over the course of the PRRSV infection, were tested for relevant cytokine induction in response to infection from the groups of P, NP and C pigs. Statistical analyses indicated that innate cytokines were increased earliest. Serum cytokine protein levels indicate that NP pigs appeared to have earlier and higher serum innate cytokine, IL-8, followed by T helper 1, IFNg, levels than the P pigs. Infected pigs exhibited high IL-1b at 14 dpi when the persistently infected pigs showed a higher concentration of IL-1b than the NP animals. Thus serum immune cytokine protein expression early after infection predicts which pigs will have persistent PRRSV infections.

The results of running the single trait model on virus level showed that persistence, day, and the day X persistence interaction were all significant. This indicated that there is indeed a significant statistical difference of virus level between pigs classified as persistent and non-persistent. The factor of day being significant indicated that virus levels varied throughout the period of examination. This makes sense since one would expect virus levels to increase at the beginning of the infection and then decrease as pigs begin to mount an immune
response. Although the initial increase in virus level is not shown, a significant decrease in both persistent and non-persistent pigs can be seen (Figure 1).

The result of the day X persistence interaction being significant indicates that there is difference between persistent and non-persistent pigs at different days (Figure 1). This result indicates that while the persistent pigs are not clearing the virus completely or at least, not as effectively as the non-persistent pigs, they are at least lowering the virus level. This is important, as it suggests that while there is some sort of immune response in the persistent pigs, it’s not as effective as the non-persistent pigs.

There was a day effect for both IL-8 and IL-1b, which means that these levels changed over time (Figure 3). However, there was no persistence or day X persistence interaction effects. This means that while the levels of the cytokines were changing, they did not appear to be affected by the persistence of a pig. Interleukin 8 and IL-1b did not appear to have an effect on persistence by day either. The day, persistence, and day X persistence interaction effects for IFNγ were all insignificant. This means that IFNγ levels did not appear to change over the period of time or the changes were undetectable and that IFNγ had no apparent affect on persistency or persistency by day.

While the individual cytokines did not appear to have an effect on persistency, they did have a significant effect on virus level, whether directly or indirectly. When the full model was run using all pigs, the main effects of IL-8, IL-1b, and day were all significant while the effects of the IL-8 X day and IFNγ X day interactions were suggestive (Table 2). This indicates that the cytokines together
have a significant effect on virus level and, since virus level was significant when run through the single trait model based on persistency, that the cytokines together could possibly have an effect on persistency or be used to predict persistency.

These results indicate that there may be immune factors that help determine why certain pigs have long term persistent PRRSV infections. Serum cytokine protein levels indicate that NP pigs could possibly have earlier and higher serum innate cytokine, IL-8, than the P pigs. This immune cytokine trend is correlated with NP pigs having lower serum and tissue viral loads; this might indicate that the NP immune response was more effective than that for P pigs and possibly enabled the NP pigs to prevent PRRSV infections to become persistent. Overall, pigs with Non-Persistent PRRSV infections appeared to have earlier and higher IL-8 levels than the pigs with Persistent infections. However, more information is needed to determine if this is actually the case or just the result of a small dataset.

The PHGC database is continuing to be updated. Now we can ask questions about potential correlations between these groups of pigs and levels of other immune parameters, e.g., neutralizing antibodies, viral levels, etc., or with genetic background (SLA class I and II genes), which was not possible previously. These studies will continue the effort to determine the role of host genetics and resistance to PRRSV.
Acknowledgements

This work was funded by the USDA Agricultural Research Service and by grants from the USDA CSREES #2004-35604-14580 PRRS CAP1, and the National Pork Board grant #07-233.

References


Figure Legend

Figure 1 – Comparison of viral clearance patterns of pigs with persistent (P) versus non-persistent (NP) PRRSV infection. Least square (LS)-mean virus level estimations for dpi 7, 14, 28, and 42 were compared. ABCDE LS Means with different letters are statistically different (p<0.05)

Figure 2. Gene Expression for immune markers for RNA prepared from tracheobronchial lymph node (TBLN) samples from PRRSV infected pig collected during the Big Pig studies. A. TBLN Immune Gene Expression (relative to controls) of PRRSV infected pigs averaged for each dpi. B. TBLN Immune Gene Expression (relative to controls) of individual PRRSV infected pigs with non-persistent (NP) and persistent (P) infections relative to control samples. * indicates statistically different expression (p<0.05).

Figure 3. Comparison of serum cytokine levels from 7 to 42 days post PRRSV infection. A. Average serum IL-8 cytokine responses of infected pigs. B. Average serum IL-8 cytokine responses of persistent (P) and non-persistent (NP) pigs. C. Average serum IL-1b cytokine responses of infected pigs. D. Average serum IL-1b cytokine responses of P and NP pigs. E. Average serum IFNγ cytokine responses of infected pigs. F. Average serum IFNγ cytokine responses of P and NP pigs. LS-means estimations for IL-8, IL-1b, and IFNγ levels for dpi 7, 14, 28, and 42 for all pigs (A, C, E) and comparison of persistent to non-persistent pigs (B, D, F). ABCD LS Means with different letters are statistically different (p<0.05).
Table 1. Characteristics for comparing pigs which are persistently infected with PRRSV.

<table>
<thead>
<tr>
<th>Pigs Total #</th>
<th>Group</th>
<th>Tissue Viral Load&lt;sup&gt;A&lt;/sup&gt; (necropsy tissue PRRSV+)</th>
<th>Serum Viral Load&lt;sup&gt;C&lt;/sup&gt; (Last Day Seropositive)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tonsil</td>
<td>Ing LN</td>
</tr>
<tr>
<td>15</td>
<td>NP&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>P&lt;sup&gt;C&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>A</sup> The Persistence status was determined based on viral RNA qPCR analyses of serum and tissues, tonsils, inguinal lymph node (IngLN) and submaxillary LN (SubLN) (Molina et al., 2008a). Tissue Viral Load noted as + if 1, 2 or 3 of Tonsil; Ing. LN; Sub. LN tested to be PRRSV + by qPCR analyses.

<sup>B</sup> NP = Non-Persistent Pigs, i.e., those with cleared infection. These pigs are defined as negative for evidence of PRRSV by viral RNA qPCR analyses of serum at 28 dpi or later and tissues at time of euthanasia (112 dpi to 202 dpi).

<sup>C</sup> P = Persistent Pigs, i.e., those with proven persistent infections (P). These pigs are defined as positive for evidence of PRRSV by viral RNA qPCR analyses of lymphoid tissues and/or tonsils at time of euthanasia (112 dpi to 202 dpi). They were serum virus qPCR positive at least until 28 dpi (average for 68 dpi); several were serum virus qPCR positive until 98-126 dpi.
Table 2. P-values of model and effects on virus level for all animals, persistent animals, and non-persistent animals

<table>
<thead>
<tr>
<th>Animals in Model</th>
<th>N#</th>
<th>Full Model</th>
<th>IL8</th>
<th>IL1b</th>
<th>IFNγ</th>
<th>Day</th>
<th>IL8*Day</th>
<th>IFN*Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>all animals</td>
<td>44</td>
<td>&lt;0.0001</td>
<td>0.0031</td>
<td>0.0099</td>
<td>0.7334</td>
<td>&lt;0.0001</td>
<td>0.0478</td>
<td>0.0883</td>
</tr>
<tr>
<td>persistent animals</td>
<td>14</td>
<td>&lt;0.0001</td>
<td>0.4416</td>
<td>0.0655</td>
<td>0.523</td>
<td>&lt;0.0001</td>
<td>0.0108</td>
<td>0.4357</td>
</tr>
<tr>
<td>non-persistent animals</td>
<td>21</td>
<td>&lt;0.0001</td>
<td>0.9923</td>
<td>0.0736</td>
<td>0.8819</td>
<td>&lt;0.0001</td>
<td>0.459</td>
<td>0.0778</td>
</tr>
</tbody>
</table>
Figure 1 – LS-mean virus level estimations for DPI 7, 14, 28, and 42 for persistent and non-persistent pigs

- **Persistent**
- **Nonpersistent**

Days After Innoculation

Virus Levels (Log)

<table>
<thead>
<tr>
<th>Days</th>
<th>Persistent</th>
<th>Nonpersistent</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>14</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>28</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>42</td>
<td>D</td>
<td>E</td>
</tr>
</tbody>
</table>
**Figure 2.** Gene Expression for immune markers for RNA prepared from IngLN in PRRSV infected pig samples collected during the Big Pig studies.
Figure 2 – LS-means estimations for IL8, IL1b, and IFNγ levels for DPI 7, 14, 28, and 42 for all pigs (a,c, and e) and persistent, non-persistent pigs (b, d, and f)
Appendix

Author’s Contributions

This section is to elaborate on the specifics of the author’s contributions to the different chapters. The work done for Chapter 2 includes creation of what is referred to as the BEAP G.U.I. and some contributions to the writing for Chapter 2 involving information about the BEAP G.U.I.

The BEAP G.U.I. was programmed with Java due to the familiarity of the programming language and the fact that Java can run on virtually any platform. The BEAP G.U.I. was designed to load a CAP3 file and parse all of the pertinent information from the file. It then displays the information pulled from the file graphically. There are many options that allow user to view sequence information in multiple formats. Sequence information can be viewed as aligned lines, aligned DNA nucleotide sequence, or aligned DNA nucleotide sequence with mismatched base pairs colored either green or red based upon the score given to the base in the CAP3 file. Users can also click on a sequence display to bring up another window that has the nucleotide sequence in the FASTA format for that specific sequence. This new window also has the option of removing that specific sequence from the aligned display in the main window.

The work done for Chapter 3 includes creation of the PHGC database, use of the R script created by Dan Nettleton to do statistical analysis and a major contribution to the writing. The PHGC database was designed based on data obtained from the “Big Pig” Project and with the idea that the database needs to be expandable to allow the inclusion of new data with relative ease. The
database was designed using MySQL due to familiarity of the designer with this DBMS as well as the ability to integrate with PHP.

A webpage interface was also designed to interact with the database. This interface was designed using HTML, PHP, and Javascript. The interface allows connection to the database using the World Wide Web, which means the database can be accessed from anywhere in the world where internet access is available. This interface also provides security implementations to help protect the data and users. Another function of the interface is to allow the entry of data into the database and retrieval of information from the database without having to know how to use SQL queries.

I worked with Joan Lunney to decide what type of analysis to do on the data from the “Big Pig” Project, which is stored in the PHGC Database. Joan told us the questions she wanted to address and we worked to create a statistical analysis to answer these questions. In this case, the question involved SLA haplotypes and the effects of virus, SNA, and cytokine levels. We contacted Dan Nettleton who created a script using R to do the analysis we felt would be appropriate. I then ran the script multiple times using different data to do a complete analysis.

For Chapter 3, I also worked on a major portion of the writing. I compiled the material and methods from information already written up by individuals who worked on the “Big Pig” Project. I then wrote the results section and worked with Joan Lunney to figure out the direction we wanted to go with the paper. With help from Joan I worked to write the abstract and discussion sections. Dan Nettleton
provided a small write-up about his script and Joan Lunney wrote the introduction.

For Chapter 4, the work done included the aforementioned work on the database, statistical analysis done using SAS, and a major contribution to the writing. The statistical analysis was done using the “Big Pig” Project data that is stored in the PHGC database. In this case, we wanted to address the issue of the effect of cytokines on persistency, which was proposed by Joan Lunney. I worked to design the statistical analysis using the SAS program to address this issue.

I once again compiled the materials and methods for the writing from information provided by those who worked on the “Big Pig” Project. I also wrote the results section. I once again worked with Joan Lunney to write the abstract and the discussion section while Joan wrote the introduction.

**Conclusions**

The goal of my master’s thesis was to create a software program to visualize CAP3 files and to create a database for the PRRS Host Genome Consortium as well as some statistical analysis on some of the data stored in the database.

One of the things I learned from creating the computer software is that the software used to do analysis can always be improved upon and that programs can be used as stepping stones to develop more tools and software. Another thing is that the software you develop can never do enough. People will want it to
have more features or complexities than the current version has and you have to draw a line at some point as the developer. I also learned that software development is more complex than I thought it was. Implementing one feature can easily disrupt how another feature works. The major point I took away was that computer software is used to create analysis programs and tools and these tools and programs are constantly advancing and becoming more complex and this is an area that is constantly evolving.

Working on the database I learned some important ideas. One of the things that I learned was that you want to have all the data available to you when you work on the design of the database. Creating a database is harder when you only get part of the information in the beginning and then have to keep changing things around to incorporate new information that should be put into the database. Another thing that I learned is that you want the people who are going to use the database to tell you what information they will be wanting entered into the database, what information they want to retrieve from the database, and what type of access they want users to have before designing the database.

When it comes to statistical analysis, I did not know what all went into designing a complete statistical analysis. One thing I learned is that compared to designing a database for data, you need to understand and know the biology behind the data more thoroughly to be able to completely comprehend what the statistics analysis involves. I also learned that even though you design a statistical analysis to test a certain hypothesis, it does not eliminate another hypothesis from being possible.
Through both the design of the database and working on the statistics I was able to learn and experience what it is like to work in collaboration with others. Communication is key when working in collaboration and requires a lot of going back and forth. Collaboration also helps to expand an analysis beyond what you personally can do because it brings in others with different expertise than yourself.

Overall, I learned a lot about what is required of research and the academic field. The possibilities of knowledge and learning are expansive and interesting. I learned that I want to continue learning and the best way to do so is to be involved in academia. Mostly, my experiences have set me up to continue on and delve deeper into the academic community.
ACKNOWLEDGEMENTS

I would like to start by thanking my major professor, Jim Reecy. Thank you for your guidance with everything over the past few years. You kept me challenged and allowed me to grow and learn new things. Thanks for all the opportunities I was given while working for you.

I would also like to thank my committee members, Leslie Miller and Sue Lamont. Thank you for the time you spent working with me to improve my thesis and improve myself. It was nice to get to work with you.

To the entire Reecy Lab Group: Thank you all. You all made my time in grad school an enjoyable experience. I would especially like to thank JR Tait and Mary Sue Mayes for their support and understanding while I worked on my thesis. Thanks Jose Rodriguez, LaRon Hughes, and Ye Cheng for being outstanding support. I would like to give special thanks to James Koltes who acted like a mentor and helped me through my graduate career. And thanks to all the undergrads who were part of the group while I was a grad student. Your antics and light hearted ways helped to break up the monotony that sometimes comes with working on a computer.

Thank you mom and dad. Your support throughout my life has given me the strength to be able to accomplish a task like this. Thank you for always believing in me and telling me to do what I love and that I can do whatever I want if I put my mind to it. Thanks.