TLR4 and IFNG are Candidate Genes for the Study of Variation in Philippine Chicken

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Abstract: Breeding programs for chickens will benefit from the identification of genes which may increase resistance to a variety of diseases because pathogenic diseases are the source of major losses for the global chicken industry. For breeding programs which involve the native Philippine chickens, screening variability in their disease resistance related genes would be extremely valuable, for very little is known about their genetic variability or disease resistance genes. Breeding in the Philippine chicken will benefit from a study that consolidates the information on genes across diseases and determines those that are involved in immune response to several diseases and have substantial data in the information repositories. 75 papers were collected providing 428 unique genes that were implicated in at least one disease. Gene ontology analysis on themes indicated that many of the gene products are localized to extracellular regions, with functions related to immune response, stress response, cell signaling and cytokines. Only 6 of the 428 genes were related to 5 or more of the 15 diseases suggesting disease responses were pathogen specific. Of these genes, TLR4 and IFNG were chosen for evaluation given the number of sequences available from different laboratories. The variation of these two genes are recommended for investigation in Philippine chicken populations.

Key Words: chicken, genetics, immunity, resistance, susceptibility

1. INTRODUCTION

The chicken industry is an important component of the economy, providing several products such as eggs and meat. The United States and Brazil are two of the major chicken product exporters. Similarly, China is also a major chicken exporter however is lesser in scale (Wen et al., 2019). In the Philippines, chickens are an important resource as they provide a source of livelihood through the selling of its meat and eggs, which can also be harvested for personal consumption. Chicken farming is a large sector of the livestock industry in the Philippines producing 1.81 million metric tons of dressed chicken and 605.79 thousand metric tons of eggs as of 2020 (Mapa, 2021).

The industry, however, has always faced challenges with disease. This has generally increased due to practices of overcrowding and the developing risk of antibiotic resistance. There have been several cases of antibiotic-resistant bacterial strains found in the gut of poultry both within the Philippines and beyond it (Sison et al., 2014; Gundran et al., 2019; Murray et al., 2021). In order to combat this, genetics-based breeding programs can be designed to create disease-resistant chickens with less reliance on antibiotics.

Gene variation has been found to confer increased disease tolerance or resistance in animals (Bishop, 2015; Kutzer & Armitage, 2016). In line with this relationship, interest has been growing in using such genetic variations in improving chicken defense against diseases. Several studies have investigated genes involved in disease response in chickens with said

objective in mind (Cheng et al.; 2008; Li et al., 2013; Wang et al., 2014; Psifidi et al., 2016).

Some genes expressed as an immune response to infection have been widely correlated with each other across diseases (Lynch et al., 2005; Keestra et al., 2013). Some genes are known to be disease specific (Young et al., 1993). Breeding for a gene that is involved in multiple diseases will be beneficial in protecting commercial populations from multiple pathogen attacks. Breeding efforts will also benefit from knowing the extent of variation in the gene across different breeds.

Indigenous breeds of livestock animals are often a source of novel genetic variants. Their adaptation to the local environment has resulted in the selection of traits that are desirable in such a location, such as resistance against common endemic diseases and tolerance to extreme temperatures (Tian et al., 2020; Weng et al., 2020).

Philippine native chickens are no exception and represent a potential reservoir for advantageous genes related to disease resistance, but they lack genetic characterization. Most genetic studies on Philippine chickens have been limited to studies on their phylogenetic relationships and genetic diversity assessments (Dominguez et al., 2016; Godinez et al., 2019).

Native chickens, however, have slowly decreased in the share of the country's total poultry inventory as they are replaced by commercial breeds (Philippine Statistics Authority, 2020). Commercial breeds have been genetically selected for superior growth rate and size as compared to native chickens (Tixier-Boichard et al., 2012). However, this selection has also resulted in exceedingly low genetic diversity (Malomane et al., 2019). Low genetic diversity decreases the resilience of populations to disease and environmental changes.

The vulnerability of commercial breeds highlights the importance of conserving indigenous Philippine breeds that are much higher in genetic diversity. These breeds may hold genetic resources important in improving the resilience of commercial breeds in the face of a growing demand for poultry, especially since some breeds of Philippine chicken have been reported to be resistant to certain pathogens according to the Domestic Animal Diversity Information System (DAD-IS, 2021). However, in order to utilize them effectively, their genetic resources must first be characterized.

The effort for characterization will benefit from focusing on specific genes that control economically attractive traits, such as disease resistance. This can be done with the aid of sequence repositories and GO analysis which can provide insights into the biological functions of the genes. Of prime importance is GenBank, a free, publicly available sequence repository which hosts data coming from all over the world. Deciding on which genes to evaluate for variability across breeds and with the Philippine population will depend on availability of sequences, the quality sources of the information and their commonality across diseases.

Gene ontology (GO) analysis, on the other hand, is done to identify shared terms found in a set of genes implicated in disease. These terms refer to different biological aspects of a gene product (Ashburner et al., 2000; Carbon et al., 2021). Terms which are found to be shared between genes implicated in a disease can be reasonably assumed to also be important in the disease.

In line with this, the study reviewed the current information on the genes related to immune response in *Gallus gallus* to determine those that are shared across different diseases, retrieved the sequences for the genes that met the criteria of numbers and quality, and evaluated their variation in preparation for a comparison with the native Philippine chicken. This study provides future researchers with a general direction for the study of variation and breeding for disease resistance in Philippine chicken genetics.

2. METHODOLOGY

2.1 Literature Search for Disease and Gene Curation

The candidate diseases and genes for disease response were collected by searching through literature on PubMed and Google Scholar. Searches were limited to genetic studies on chickens, and search terms used included keywords such as "disease resistance", "disease response", and the names of diseases in poultry. Diseases included in the study were selected based on the availability of published papers that discussed genes implicated in the response or resistance of chickens to diseases. Another criterion considered is that only publications that discussed differentially expressed genes (DEGs) and SNPs were included. Publications that discussed quantitative trait loci (QTLs) were excluded.

2.2 Gene Analysis

Gene ontologies were developed using g:Profiler, an online functional enrichment analysis tool. The tool g:Profiler was chosen over other functional enrichment analysis tools due to its ability to recognize several types of gene identifiers (Raudvere et al., 2019).

Collected genes from literature were scored based on the presence of studies that showed that the variation of these genes affected the response and/or resistance to a certain disease. Two types of variation were considered: structural variation, i.e., the presence of polymorphisms in the gene, and variation in gene expression. The list of genes by disease were tabulated in Microsoft Excel and the same program was used to procedurally record the number of diseases each gene was involved in. Genes were given a point for every disease they were confirmed to be involved in based on the literature. Points were totaled and genes were ranked from highest to lowest. From the ranking, the top 5 genes were taken as genes of interest for further study.

2.3 Haplotype Analysis

One hundred forty-two (142) and one hundred fifty-two (152) published sequences of Gallus gallus TLR4 and IFNG, respectively, were retrieved from GenBank**.** These sequences came from different regions and laboratories globally.

The sequences were trimmed and aligned in MEGA version X using the MUSCLE codon algorithm (Kumar et al., 2018). The alignments used for Gallus gallus IFNG and TLR4 were used to identify SNPs and haplotypes. DnaSP was used to detect haplotypes (Rosaz et al., 2017). SNP-sites were used to tabulate the SNPs of each haplotype (Page et al., 2016). A R-based workflow by Toparslan et al. (2020) was used to visualize haplotype data and networks.

Sequences, accession codes, and other relevant data for this study may be found in Hernandez & Suficiencia (2022).

3. RESULTS AND DISCUSSION

3.1 Nature of Diseases in the Study

Chickens have around 30 or so prominent diseases globally, and this is comparable to other raised livestock. Of these 30 diseases, 15 are included in this study. The list of diseases was obtained from 73 unique papers. Half of the diseases in this list are bacterial in origin. (Figure 3.1).

Figure 3.1 Pathogen types across diseases

Gastrointestinal, lymphatic, and respiratory systems are the most commonly affected systems. Detailed information on the chosen diseases, including the nature of the pathogen, transmission, pathogenesis, and clinical manifestations are detailed in Hernandez & Suficiencia (2022). Majority of the diseases have been reported in the Philippine chicken population, except for lymphoid leukosis and necrotic enteritis (Nagaoka et al., 1994; Tateyama et al., 1999; Sison et al., 2014; Tapdasan et al., 2015; Caceres et al., 2017; Peters et al., 2018; Bautista & Mendoza, 2018; Elumba et al., 2018; Ybañez et al., 2018). Though lymphoid leukosis and necrotic enteritis have not been reported for Philippine chickens, these diseases are included in the research given the possibility of future infection and the economic burden should future infections occur.

3.2 Gene Ontology

A total of 523 gene names were identified to be implicated in disease resistance or response of the 15

diseases chosen. Four hundred twenty-eight (428) genes were left after pruning duplicate genes between diseases, redundant gene identifiers (i.e., two different gene symbols referring to the same gene). Genes with inconsistent records in databases were also excluded. The abundance of genes detected in the literature review indicate the potential variety in response mechanisms to a pathogen and the complexity of the gene expression processes that are involved with disease in chickens.

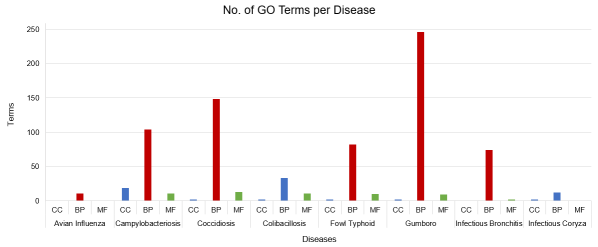

Figure 3.2 Number of GO Terms by Category of the First 8 Diseases

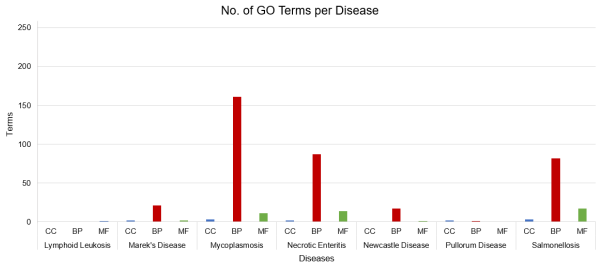

Figure 3.3 Number of GO Terms by Category of the Remaining 7 Diseases

Gene ontology analysis was done to identify shared terms found in a set of genes implicated in disease for characterization. Majority of these genes were involved in the immune system. It is also important to note the variation in the abundance of significant terms per disease (Figure 3.2 and 3.3). This may be due to two reasons. First, the genes implicated in a disease may be considerably unique from each other, thereby reducing the number of shared terms between them. On the other hand, it may be that the terms the genes share truly are limited in number, due to their similarity. A more detailed gene ontology analysis of the data can be found in Hernandez & Suficiencia (2022).

3.3 Gene Scoring

Of the 428 genes culled from the 73 papers, only 55 or 12.9% were found to be common in at least 2 diseases. Only 6 genes or 1.4% of total genes were involved in 5 or more diseases (Table 3.1). The full scoring table for the top 55 genes by disease can be found in Hernandez & Suficiencia (2022).

Table 3.1 Top 10 Genes Shared Across Diseases

Gene Symbol	Gene Name	Number of Diseases Implicated In
IL8L2	**Interleukin 8-like 2**	10
IL6	**Interleukin 6**	7
IL10	**Interleukin 10**	5
IL1B	**Interleukin 1 Beta**	5
IFNG	**Interferon Gamma**	5
TLR4	**Toll-Like Receptor 4**	5
CCL4	**C-C Motif Chemokine Ligand 4**	4
IL12B	**Interleukin 12 Beta**	4
AvBD1	**Avian Beta-Defensin 1**	3
AvBD4	**Avian Beta-Defensin 4**	3

There are a few key genes that may be studied for further analysis due to their involvement in more than two diseases. The relative scarcity of genes involved in several diseases may be due to the presence of different types of pathogens considered in the study (i.e., viral, bacterial, protozoan). Pathogen types, whether they be bacterial, viral, or parasitic, have differing mechanisms of infecting chickens and as such would cause different responses within the host. The number of pathogens and mechanisms for response will require an array of genes/pathways to be investigated for immune response.

Targeting genes involved in multiple diseases as opposed to those known only to affect one disease may be beneficial in improving overall resistance to multiple diseases but will generally be less effective for specific diseases.

Such genes may be utilized in breeding programs of chicken lines to improve disease resistance. Currently, it is the paradigm in commercial breeding programs to mainly focus on improving economic traits like meat quality and shortening time to full maturity (Saxena & Kolluri, 2018; Okeno, Kahi, & Peters, 2013), with less focus on disease response and resistance. Indeed, there is no known breeding program using candidate genes involved in disease resistance. However, the growing threat of epidemics may encourage new breeding programs based on enhancing immunity.

Table 3.2 Top 6 Genes and Number of Published Sequences in GenBank

Gene	No. of Published Sequences in GenBank
IL8L2	1
IL6	9
IL10	4
IL1B	145
IFNG	153
TLR4	142

Only two genes were selected for further analysis, namely IFNG and TLR4 due to the substantial number of published sequences (Table 3.2). The remainder of the top 5 genes were either lacking in published sequences or in the case of IL1B, only had sequences originating from a single paper (Downing et al., 2009).

In Philippine native chickens, there must then be more in-depth investigations in the genetic variation of these immune-response genes. In terms of breeding programs involving native Philippine chickens, on one hand, breeding could be done with multiple genes conferring attractive disease response traits for a maximal increase in disease resistance. On the other hand, breeding could also be done with focus to a single gene with an attractive disease response trait, although this would have a lower impact on disease resistance improvement.

3.4 Haplotype Network

Toll-like receptor 4 (TLR4)

TLR4 has 94 haplotypes with 30 SNPs spread across the gene. Among the haplotypes, haplotype 40 and 41 have a bootstrap value between 70-85%. Haplotypes 7 and 4 have the next highest bootstrap value between 50-70% (Figure 3.4). The remaining haplotypes have a low bootstrap value of below 50%. Low bootstrap values mean that there is low confidence in the relationship among haplotypes. Generally, branches with bootstrap values of >70% can be regarded with high confidence as being true in nature (Hillis & Bull, 1993).

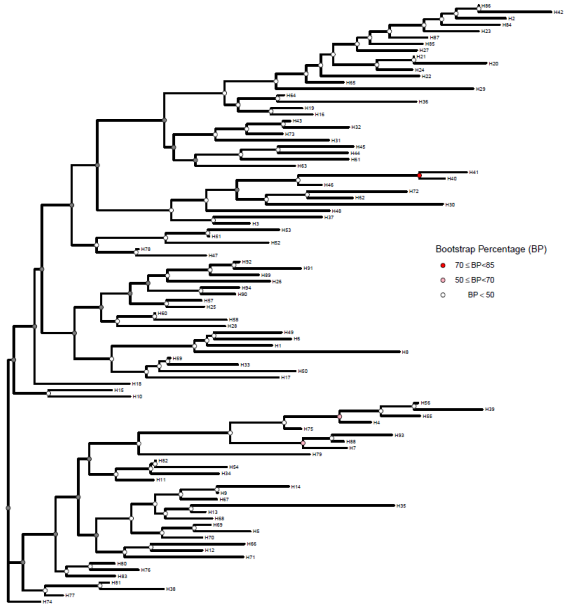

Figure 3.4 *Gallus gallus* TLR4 Haplotype Tree

There exists 94 haplotypes for TLR4 implying different variants of the gene. However, the low bootstrap values of the majority of the haplotypes should be noted. Only 4 haplotypes out of 94 had high bootstrap values This could be indicative of silent mutations which do not affect the protein produced hence it is not significant but further tests should be done to confirm this.

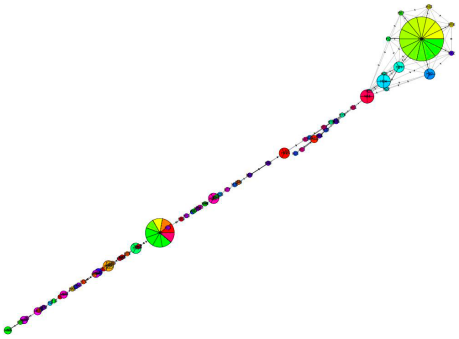

Figure 3.5 *Gallus gallus* TLR4 Haplotype Network

A haplotype network of TLR4 was developed to visualize the relationship of its haplotypes (Figure 3.5). The 94 circles represent the 94 haplotypes detected within the sequences used in the study. Among the haplotypes, haplotype 86 has the most individuals having 14 sequences and represents the origin of the haplotypes. Majority of the haplotypes for TLR4 had 1-3 sequences under them. In terms of branching, the haplotype with the most branches is also haplotype 86 with 7 haplotypes branching out from it.

There is an evident linear progression in haplotypes of TLR4 which implies a fast mutation rate for TLR4. Evolutionary rates may be associated with its function in pathogen recognition. TLR4 in chickens recognizes lipopolysaccharides of gram-negative bacteria leading to an immune response (Nawab et al., 2019). Lipopolysaccharides are examples of a PAMP recognized by pattern recognition receptors (PRRs) such as TLR4 (Neerukonda & Katneni, 2020). PAMPs associated with TLRs tend to experience selective sweeps which TLRs must adapt to accordingly (Downing et al., 2010).

Interferon Gamma (IFNG)

The IFNG neighbor-joining tree (Figure 3.6) indicates that there are 7 haplotypes present among the sequences. All branches have low bootstrap percentages with none having a significant bootstrap value. This indicates that there is low confidence in the relationships between haplotypes.

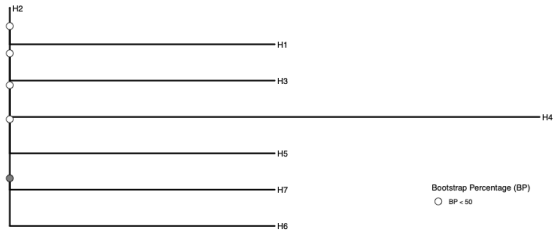

Figure 3.6 *Gallus gallus* IFNG Haplotype Tree

Figure 3.7 shows the haplotype network of Gallus gallus IFNG. The Gallus gallus IFNG gene had 7 SNPs and 7 haplotypes. The majority of sequences belonged to Haplotype 2. Haplotype 2 is the probable origin of the remaining six haplotypes given that all branch out from said haplotype.

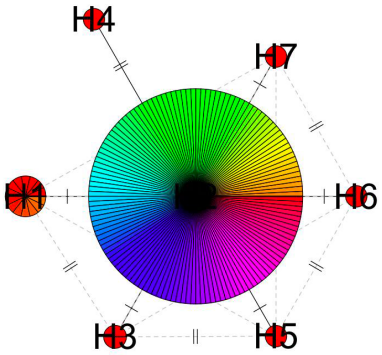

Figure 3.7 *Gallus gallus* IFNG Haplotype Network

The radiated branching character in the haplotype network of IFNG contrasts with the linear one of TLR4. This is indicative of slower mutation rates or less tolerance to mutation. Mutation rates in IFNG may be slower given the essential functions of IFNG in the

avian immune system (Downing et al., 2009). IFNG links the responses of the innate and adaptive immune system by promoting MHC II expression, and regulates the functions of macrophages, natural killer cells, and B cells (Fensterl & Sen, 2009). Given the wide range of functions that IFNG has, even slight mutations in its sequence could cause its effectivity in carrying out these functions to decrease.

It would be interesting to determine how the Philippine chicken TLR4 and IFNG genes would figure in the patterns demonstrated by the samples already studied. Backyard-raised native chickens in the Philippines often scavenge for food and water. When given feeds, they are broadcasted into the ground which gives them more exposure to potential pathogens (Naldo et al., 2021; Dusaran & Pabulayan, 2012). This may result in the selection of more disease-resistant phenotypes controlled by these two genes.

4. CONCLUSIONS

Diseases in chickens make up billions in economic costs globally. This is especially experienced in the case of developing countries like the Philippines where small-scale poultry farmers have lacking access to information, treatment, and prophylaxis. Native chickens have also been lacking in genetic characterization despite their potential for genetic resources. Thus, this study aimed to find important genes implicated in disease response in chickens, their characterization, and variability for use in the Philippine chicken.

This study reviewed over 73 unique papers on genes implicated in disease response across 15 selected diseases of global economic importance. Four hundred twenty-eight (428) unique genes were found and 55 of these were implicated in two or more diseases. This implied the diversity of pathogen processes and the need for the enhancement of an array of genes/pathways.

Functional enrichment analysis provided insights into their shared biological terms of the genes helping in their characterization. The majority of the terms were involved in the immune system process.

Of the top 6 genes, TLR4 and IFNG were chosen for haplotype analysis. Haplotype analysis showed the differing mutation rates between TLR4 and IFNG. TLR4 is experiencing higher rates due to its function in pattern recognition..

It is further recommended to test the remaining genes in the top 6 for their variation which would require an extensive collection of samples and sequences as online databases lack this with the exception of IL1B. In addition, it is recommended that studies be done per pathogen type (viral/bacterial/parasitic), to garner more specific insights to genes involved in their disease response. Finally, lab samples from Philippine native chickens should also be tested for any variations in these genes.

5. ACKNOWLEDGMENTS

We would like to thank our defense panelists Dr. Chona, Dr. Redillas, and Dr. Ples for providing recommendations and constructive criticism.

6. REFERENCES

Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., Davis, A. P., Dolinski, K., Dwight, S. S., Eppig, J. T., Harris, M. A., Hill, D. P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J. C., Richardson, J. E., Ringwald, M., Rubin, G. M., & Sherlock, G. (2000). Gene Ontology: tool for the unification of biology. Nature Genetics, 25(1), 25. https://doi.org/10.1038/75556

Bautista, V. A. L. M., & Mendoza, B. C. (2018). Serogroup, pathotype and multiple drug resistance of Escherichia coli strains isolated from the cloaca of layer chickens in San Jose, Batangas, Philippines. Philipp Sci Lett, 11, 69-77.

Bishop S. C. (2015). Genetic resistance to infections in sheep. Veterinary microbiology, 181(1-2), 2–7. https://doi.org/10.1016/j.vetmic.2015.07.013

Caceres, A.B., Umali, D.V., Bernardo, F.A.E.M., & Katoh, H. (2017) Molecular characterization of Infectious Bursal Disease virus from gamefowls in selected

areas in CALABARZON, Philippines. International J. of Life Sciences, 5 (4): 491-498.

Carbon, S., Douglass, E., Good, B. M., Unni, D. R., Harris, N. L., Mungall, C. J., Basu, S., Chisholm, R. L., Dodson, R. J., Hartline, E., Fey, P., Thomas, P. D., Albou, L.-P., Ebert, D., Kesling, M. J., Mi, H., Muruganujan, A., Huang, X., Mushayahama, T., … Elser, J. (2021). The Gene Ontology resource: enriching a GOld mine. Nucleic Acids Research, 49(D1), D325–D334. https://doi.org/10.1093/nar/gkaa1113

Cheng, H., Niikura, M., Kim, T., Mao, W., MacLea, K. S., Hunt, H., Dodgson, J., Burnside, J., Morgan, R., Ouyang, M., Lamont, S., Dekkers, J., Fulton, J., Soller, M., & Muir, W. (2008). Using integrative genomics to elucidate genetic resistance to Marek's disease in chickens. Developments in biologicals, 132, 365–372. https://doi.org/10.1159/000317187

DAD-IS. (2021). Domestic Animal Diversity Information System (DAD--IS) Breed Data Sheet, Food and Agriculture Organization of the United Nations. Retrieved from http://www.fao.org/dad-is/browse-by-country-and-species/en/

Dominguez, J., Salces, A., & Yebron, M. (2016). Genetic variation and relationships among Visayan native chickens genetic groups Boholano and Darag (Gallus gallus L.) Philippine Journal of Veterinary and Animal Sciences, 42, 8-15.

Downing, T., Lloyd, A. T., O'Farrelly, C., & Bradley, D. G. (2010). The differential evolutionary dynamics of avian cytokine and TLR gene classes. Journal of immunology (Baltimore, Md. : 1950), 184(12), 6993–7000. https://doi.org/10.4049/jimmunol.0903092

Downing, T., Lynn, D. J., Connell, S., Lloyd, A. T., Bhuiyan, A. F. H., Silva, P., Naqvi, A. N., Sanfo, R., Sow, R. S., Podisi, B., O'Farrelly, C., Hanotte, O., & Bradley, D. G. (2009). Contrasting evolution of diversity at two disease-associated chicken genes. Immunogenetics, 61(4), 303–314. https://doi.org/10.1007/s00251-009-0359-x

Dusaran R.N., & Pabulayan, R.A. (2012). Production Practices of the Native Chicken Growers in Western Visayas. Patubas 7(1):40-65.

Elumba, Z. S., Allera, M. L. M., & Taganas, R. R. R. (2018). Occurrence and Antibiotic Sensitivity of Escherichia coli and Salmonella spp. in Retail Chicken Meat at Selected Markets in Valencia City, Bukidnon, Philippines. Asian Journal of Biological and Life Sciences, 7(2), 53. http://dx.doi.org/10.5530/ajbls.2018.7.4

Fensterl, V., & Sen, G. C. (2009). Interferons and viral infections. In BioFactors (Vol. 35, Issue 1, pp. 14–20). https://doi.org/10.1002/biof.6

Godinez, C. J. P., Nishibori, M., Matsunaga, M., & Espina, D. M. (2019). Phylogenetic studies on red junglefowl (Gallus gallus) and native chicken (Gallus gallus domesticus) in Samar Island, Philippines using the mitochondrial DNA D-Loop region. The Journal of Poultry Science, 56(4), 237–244. https://doi.org/10.2141/jpsa.0180131

Gundran, R. S., Cardenio, P. A., Villanueva, M. A., Sison, F. B., Benigno, C. C., Kreausukon, K., Pichpol, D., & Punyapornwithaya, V. (2019). Prevalence and distribution of blaCTX-M, blaSHV, blaTEM genes in extended- spectrum β- lactamase- producing E. coli isolates from broiler farms in the Philippines. BMC veterinary research, 15(1), 227. https://doi.org/10.1186/s12917-019-1975-9

Hillis, D. M., & Bull, J. J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic biology, 42(2), 182-192. https://doi.org/10.1093/sysbio/42.2.182

Hernandez, J. T., & Suficiencia, Z. L. (2022). Evaluating variability in interferon gamma and toll like receptor 4 in the chicken (gallus gallus, linnaeus, 1758) for comparison of known breeds with the

native Philippine chicken. Retrieved from https://animorepository.dlsu.edu.ph/etdb_bio/9

Hillis, D. M., & Bull, J. J. (1993). An Empirical Test of Bootstrapping as a Method for Assessing Confidence in Phylogenetic Analysis. In Systematic Biology (Vol. 42, Issue 2, pp. 182–192). Oxford University Press (OUP). https://doi.org/10.1093/sysbio/42.2.182

Keestra, A. M., de Zoete, M. R., Bouwman, L. I., Vaezirad, M. M., & van Putten, J. P. (2013). Unique features of chicken Toll-like receptors. *Developmental and comparative immunology*, *41*(3), 316–323. https://doi.org/10.1016/j.dci.2013.04.009

Kutzer, M. A., & Armitage, S. A. (2016). Maximising fitness in the face of parasites: a review of host tolerance. Zoology (Jena, Germany), 119(4), 281–289. https://doi.org/10.1016/j.zool.2016.05.011

Li, D. F., Lian, L., Qu, L. J., Chen, Y. M., Liu, W. B., Chen, S. R., Zheng, J. X., Xu, G. Y., & Yang, N. (2013a). A genome-wide SNP scan reveals two loci associated with the chicken resistance to Marek's disease. Animal genetics, 44(2), 217–222. https://doi.org/10.1111/j.1365-2052.2012.02395.x

Lynch, N. J., Khan, S.-H., Stover, C. M., Sandrini, S. M., Marston, D., Presanis, J. S., & Schwaeble, W. J. (2005). Composition of the Lectin Pathway of Complement inGallus gallus: Absence of Mannan-Binding Lectin-Associated Serine Protease-1 in Birds. In The Journal of Immunology (Vol. 174, Issue 8, pp. 4998–5006). The American Association of Immunologists. https://doi.org/10.4049/jimmunol.174.8.4998

Malomane, D. K., Simianer, H., Weigend, A., Reimer, C., Schmitt, A. O., & Weigend, S. (2019). The SYNBREED chicken diversity panel: a global resource to assess chicken diversity at high genomic resolution. BMC Genomics, 20(1). https://doi.org/10.1186/s12864-019-5727-9

Mapa, D.S. (2021) Chicken Situation Report: January-December 2020. Philippines Statistics Authority.https://psa.gov.ph/sites/default/files/3_SR%20Chicken%20Annual%20Situation%20Report_signed.pdf

Murray, M., Salvatierra, G., Dávila-Barclay, A., Ayzanoa, B., Castillo-Vilcahuaman, C., Huang, M., Pajuelo, M. J., Lescano, A. G., Cabrera, L., Calderón, M., Berg, D. E., Gilman, R. H., & Tsukayama, P. (2021). Market Chickens as a Source of Antibiotic-Resistant Escherichia coli in a Peri-Urban Community in Lima, Peru. In Frontiers in Microbiology (Vol. 12). Frontiers Media SA. https://doi.org/10.3389/fmicb.2021.635871

Nagaoka, K., De Mayo, A., Takagi, M., & Ohta, S. (1994). Characterization of Haemophilus paragallinarum isolated in the Philippines. Journal of Veterinary Medical Science, 56(5), 1017-1019. https://doi.org/10.1292/jvms.56.1017

Naldo, M. A., Agbisit, E. M., Salces, A. J., Dondi, M., Arboleda, M., & Magpantay, V. A. (2021). SMALL-SCALE NATIVE CHICKEN PRODUCTION SYSTEMS IN MOUNTAIN BARANGAYS OF AYUNGON, NEGROS ORIENTAL, PHILIPPINES. In Philipp J Vet Anim Sci (Vol. 47, Issue 1).

Nawab A, An L, Wu J, Li G, Liu W, Zhao Y, Wu Q, Xiao M. Chicken toll-like receptors and their significance in immune response and disease resistance. Int Rev Immunol. 2019;38(6):284-306. doi: 10.1080/08830185.2019.1659258. Epub 2019 Oct 30. PMID: 31662000.

Neerukonda, S. N., & Katneni, U. (2020). Avian Pattern Recognition Receptor Sensing and Signaling. Veterinary Sciences, 7(1), 1–41. https://doi.org/10.3390/VETSCI7010014

Okeno, T. O., Kahi, A. K., & Peters, K. J. (2013). Evaluation of breeding objectives for purebred and crossbred selection schemes for adoption in

indigenous chicken breeding programmes. British Poultry Science, 54(1), 62–75. doi:10.1080/00071668.2013.7644

Philippine Statistics Authority. (2020). Livestock and poultry statistics of the Philippines, 2015-2019. Retrieved from https://psa.gov.ph/content/livestock-and-poultry-statistics-philippines.

Psifidi, A., Banos, G., Matika, O., Desta, T. T., Bettridge, J., Hume, D. A., Dessie, T., Christley, R., Wigley, P., Hanotte, O., & Kaiser, P. (2016). Genome-wide association studies of immune, disease and production traits in indigenous chicken ecotypes. Genetics, selection, evolution : GSE, 48(1), 74. https://doi.org/10.1186/s12711-016-0252-7

Raudvere, U., Kolberg, L., Kuzmin, I., Arak, T., Adler, P., Peterson, H., & Vilo, J. (2019). g:Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). Nucleic Acids Research, 47(W1), W191–W198. https://doi.org/10.1093/NAR/GKZ369

Saxena, V. K. , & Kolluri, G. (2018). Selection Methods in Poultry Breeding: From Genetics to Genomics. In (Ed.), Application of Genetics and Genomics in Poultry Science. IntechOpen. https://doi.org/10.5772/intechopen.77966

Sison, F. B., Chaisowwong, W., Alter, T., Tiwananthagorn, S., Pichpol, D., Lampang, K. N., Baumann, M. P., & Gölz, G. (2014). Loads and antimicrobial resistance of Campylobacter spp. on fresh chicken meat in Nueva Ecija, Philippines. Poultry science, 93(5), 1270–1273. https://doi.org/10.3382/ps.2013-03791

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular biology and evolution*, *35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Tapdasan, E. P., Wongsathapornchai, K., Chanachai, K., Benigno, C. C., Gundran, R. S., Daguro, L. I., & Lapiz, S. M. D. (2016). Outbreak of Newcastle Disease among Recipients of the Poultry Dispersal Program in Bohol, Philippines, February 2013. OSIR Journal, 8(1), 1-6.

Tateyama, S., Molina, H. A., Uchida, K., Yamaguchi, R., & Manuel, M. F. (2000). An epizootiological survey of necropsy cases (1993-1997) at University of the Philippines. Journal of Veterinary Medical Science, 62(4), 439-442. https://doi.org/10.1292/jvms.62.439

Tian, S., Zhou, X., Phuntsok, T., Zhao, N., Zhang, D., Ning, C., Li, D., & Zhao, H. (2020). Genomic Analyses Reveal Genetic Adaptations to Tropical Climates in Chickens. IScience, 23(11), 101644. https://doi.org/10.1016/J.ISCI.2020.101644

Tixier-Boichard, M., Leenstra, F., Flock, D. K., Hocking, P. M., & Weigend, S. (2012). A century of poultry genetics. World's Poultry Science Journal, 68(2), 307–321. https://doi.org/10.1017/s0043933912000360

Wang, Y., Lupiani, B., Reddy, S. M., Lamont, S. J., & Zhou, H. (2014). RNA-seq analysis revealed novel genes and signaling pathway associated with disease resistance to avian influenza virus infection in chickens. Poultry science, 93(2), 485–493. https://doi.org/10.3382/ps.2013-03557

Wen, X., Li, L., Sun, S., He, Q., & Tsai, F. S. (2019). The Contribution of chicken products' export to economic growth: evidence from China, the United States, and Brazil. Sustainability, 11(19), 5253. https://doi.org/10.3390/su11195253

Weng, Z., Xu, Y., Li, W., Chen, J., Zhong, M., Zhong, F., Du, B., Zhang, B., & Huang, X. (2020). Genomic variations and signatures of selection in Wuhua yellow chicken. PLOS ONE, 15(10), e0241137. https://doi.org/10.1371/JOURNAL.PONE.0241137

Ybañez, R., Resuelo, K., Kintanar, A., & Ybañez, A. P. (2018). Detection of gastrointestinal parasites in

small-scale poultry layer farms in Leyte, Philippines. Veterinary world, 11(11), 1587–1591. https://doi.org/10.14202/vetworld.2018.1587-1591

Young, J. A., Bates, P., & Varmus, H. E. (1993). Isolation of a chicken gene that confers susceptibility to infection by subgroup A avian leukosis and sarcoma viruses. *Journal of virology*, *67*(4), 1811–1816. https://doi.org/10.1128/JVI.67.4.1811-1816.1993