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Identification of variants in *GBP1* and *GBP5* Genes associated with susceptibility and resistance to porcine reproductive and respiratory syndrome in Uruguayan Creole pigs

Identificación de variantes en los genes *GBP1* y *GBP5* asociados a resistencia y susceptibilidad al síndrome reproductivo y respiratorio porcino en cerdos criollos de Uruguay

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ABSTRACT

Porcine reproductive and respiratory syndrome (PRRS) is a viral disease that affects pigs, causing significant economic losses in the global swine industry due to reproductive and respiratory problems. The causative agent of PRRS is the PRRS virus (PRRSV), primarily transmitted through direct or indirect contact via respiratory or oral routes. Despite biosecurity measures, monitoring, and vaccination, there is currently no fully effective vaccine against this virus. Research has identified a quantitative trait locus on chromosome 4 associated with PRRSV resistance. This locus includes genetic polymorphisms rs80800372 (WUR) and rs340943904 in the *GBP1* and *GBP5* genes, respectively. PRRSV has been detected in South America, including Uruguay in 2017. In Uruguay, the Pampa Rocha pig is the only breed of Creole pigs and is at risk due to its small population. In this context, the objective was assessing genetic variability in the Pampa Rocha breed for relevant variables related to PRRS resistance. The study determined the genotype for these variants using the end-point PCR technique, followed by Sanger sequencing. In the study, corresponding alleles were identified for each variable of interest, with allele frequencies of 0.825 for the A allele and 0.175 for the G allele in rs80800372 (WUR), and 0.825 for the G allele and 0.175 for the T allele in rs340943904. The variants are in Hardy Weinberg equilibrium and there is a linkage disequilibrium between them. The study highlights an increase in the frequency of favorable alleles related to PRRSV resistance in Pampa Rocha creole pigs. These findings underscore the importance of using molecular markers to identify PRRS-resistant animals, which could be beneficial for both pig production and animal welfare.

Key words: genetic resistance; creole pigs, PRRS

RESUMEN

El síndrome reproductivo y respiratorio porcino (PRRS) es una enfermedad viral que afecta a cerdos, provocando problemas reproductivos y respiratorios que causan pérdidas económicas significativas en la industria porcina mundial. El virus PRRSV es el agente responsable, transmitido principalmente por contacto directo o indirecto a través de vías respiratorias u orales. Aunque el control de este virus implica medidas de bioseguridad, monitoreo y vacunación, no existe actualmente una vacuna totalmente eficaz. Investigaciones han identificado un locus de rasgo cuantitativo en el cromosoma 4 asociado con la resistencia al PRRSV, que incluye a los polimorfismos rs80800372 (WUR) y rs340943904 en los genes *GBP1* y *GBP5* respectivamente. El PRRSV ha sido detectado en América del Sur, incluido Uruguay en el año 2017. En Uruguay, los cerdos Pampa Rocha son la única raza de cerdos criollos y se encuentran en riesgo debido a su baja población. En este contexto, se plantea evaluar la variabilidad genética en esta raza para las variables de interés, relacionadas con la resistencia al PRRS. Para determinar los genotipos se utilizó la técnica de PCR en tiempo final, seguida de secuenciación Sanger. Se identificaron los alelos correspondientes para cada variable, con frecuencias de 0,825 para el alelo A y 0,175 para el alelo G en rs80800372 (WUR), y de 0,825 para el alelo G y 0,175 para el alelo T en rs340943904. Ambas variantes se encuentran en equilibrio de Hardy Weinberg y presentan desequilibrio de ligamiento. El estudio destaca un aumento en la frecuencia de los alelos favorables en los genes *GBP1* y *GBP5* relacionados con la resistencia al PRRSV, en los cerdos Pampa Rocha. Estos hallazgos subrayan la importancia de utilizar marcadores moleculares para identificar animales resistentes al PRRS, lo cual podría ser beneficioso para la producción porcina y el bienestar animal.

Palabras clave: resistencia genética; cerdos criollos, PRRS

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INTRODUCTION

The porcine reproductive and respiratory syndrome (PRRS) is a viral disease that causes reproductive and respiratory complications in pigs (*Sus scrofa ferus*), significantly impacting animal welfare and resulting in considerable economic losses globally within the swine industry. Reproductive consequences include spontaneous abortions in females, premature births, and a decline in the quality of semen from boars. Meanwhile, respiratory issues reduce the growth rate of pigs throughout the fattening stage [1, 2].

The causative agent of this disease is the PRRS virus (PRRSV), which is a single-stranded positive-sense RNA virus belonging to the *Betaarterivirus* genus and the *Arteriviridae* family. This virus is classified into two genotypes: PRRSV-1 (European) and PRRSV-2 (North American) [3, 4].

Transmission of PRRSV can occur through direct or indirect contact, primarily via respiratory or oral routes, penetrating mucous membranes or even percutaneously. The virus can be transmitted airborne, during mating or insemination, through ingestion, contact, or inoculation [5]. During gestation, the virus has the ability to cross the placental barrier and infect embryos, potentially leading to the most severe clinical manifestation of the disease at the end of gestation. This is characterized by abortions, premature births, mummification, and the birth of weak and congenitally infected piglets, leading to high mortality before weaning [6].

PRRSV control involves several key aspects, including early diagnosis, continuous monitoring, implementation of biosecurity measures, and proper herd management and vaccination [5]. Currently, there is no fully effective vaccine against PRRSV due to the virus's genetic and antigenic variations, as well as its ability to evade the host immune response. Therefore, it is crucial to explore alternative control strategies, with genetic improvement of pigs being one option [7, 8]. In this regard, different pig breeds exhibit varying levels of resistance to PRRSV, emphasizing the importance of studying genetic factors to enhance pig resistance to this disease. Such efforts would contribute to animal welfare and mitigate the associated economic losses [4, 9].

Through genome-wide association studies, a quantitative trait locus (QTL) has been identified on chromosome 4 (SSC4), associated with host resistance to PRRSV, weight gain, and viral load [10]. Within the single nucleotide polymorphisms (SNPs) identified in this region, rs80800372 (known as WUR) occurs in the *GBP1* gene, and rs340943904 in the *GBP5* gene [11, 12]. Since the identification of this QTL, the effect of the WUR variant has been associated with increased weight and viral load following PRRS infection [10]. Additionally, it has been linked to the host response to PRRSV infection, PRRS vaccination, and coinfection with PRRSV and porcine circovirus type 2b [13]. Subsequent research by Koltes *et al.* [12] revealed that the candidate gene is the one encoding guanylate-binding protein 5 (*GBP5*), located in the region surrounding WUR.

The rs80800372 variant in *GBP1* corresponds to an A/G mutation in the 3' untranslated region (3'UTR), with G being the favorable allele, while rs340943904 in *GBP5* is a G/T variant at a splice site, with the T allele being favorable [14].

PRRS was first identified in the late 1980s in North America and Europe [15]. In South America, PRRSV has been reported in Bolivia, Chile, Colombia, Peru, Venezuela, Ecuador and Uruguay [16]. In Uruguay, the first detection of the virus was carried out by Ramos *et al.* [17], identifying the circulation of PRRSV type 2. This study included a retrospective serological analysis suggesting that the virus may have been present in the country since 2011.

Pig production in Uruguay, while economically less significant, plays a crucial role in supporting low-income producers [18]. In this context, local zoogenetic resources become more important due to their better adaptation to local conditions and their production capacity with lower requirements. These animals are commonly utilized in small-scale traditional subsistence systems, playing a fundamental role in ensuring food security [19]. In Uruguay, the Pampa Rocha breed represents the only creole pig breed [20]. These pigs stand out for the qualities of their females, including characteristics such as prolificacy, ability to consume pastures, milk production, and productive longevity [21]. However, the current number of animals of this breed is unknown, posing a risk to their conservation.

To date, no studies have been conducted in Uruguay evaluating the genetic resistance/susceptibility to PRRSV in Pampa Rocha creole pigs. Based on this gap, the objective is to determine the genotypes for the variants rs80800372 (in *GBP1*) and rs340943904 (in *GBP5*) in this local pig breed in Uruguay. This preliminary investigation is relevant for enhancing the understanding of the genetic variability present in Pampa Rocha pigs and contributing to their conservation.

MATERIALS AND METHODS

Twenty DNA samples from pigs were utilized in this study, including 14 Pampa Rocha, three Pampa Rocha-Duroc hybrids, one Large White, one Duroc, and one Pietrain. These samples are part of the DNA bank at the Academic Unit of Animal Genetics and Improvement within the Faculty of Veterinary Medicine at the University of the Republic (Udelar) in Montevideo, Uruguay. These animals come from a rescue center for the conservation of the breed.

Genotypes were determined using the end-point PCR technique, followed by Sanger sequencing [22]. Specific primers were designed using the Primer BLAST tool [23].

Amplification was performed using a Multigene II equipment (Labnet International, Inc. USA). TABLE I provides details of the primers used and the amplification conditions for the regions containing both variants.

The amplification results were analyzed using agarose gel electrophoresis (1% agarose gel stained with Goodview Nucleic Acid Stain) in 1× TBE buffer. Electrophoresis was conducted using an HU13 MIDI Horizontal Gel electrophoresis system (Scie-plas, Great Britain) and a POWER PAC 3000 power supply (Bio-Rad, USA). The resulting bands were visualized under UV light using a BIOSENS SC805-BIOTOP instrument (Shanghai Bio-Tech Co. Ltd. China). Amplicon sequencing was performed in a sequencer ABI 3500 (Thermofisher, USA) by the company Genexa (Montevideo, Uruguay). Sequence analysis and determination of genotypes for the studied variants were carried out through alignment using the BioEdit program [24]. Reference sequences for porcine *GBP1* and *GBP5* genes were retrieved from the Ensembl database (ensembl.org). Allelic and genotypic frequencies, as well as the calculation of Fis values according to Weir and Cockerham [25], were determined using the GENETIX V 4.05 program [26]. The Hardy-Weinberg exact test was performed for each locus using the Genepop version 4.7.5 web tool [27, 28]. Finally, linkage disequilibrium between the variants was determined according to Black and Krafsur [29] in the GENETIX V 4.05 program [26].

TABLE I
Analyzed variants and amplification conditions

Variant	Gene	Primers	Amplification conditions	Amplicon size
rs80800372 (WUR10000125)	*GBP1*	F: GGAATGCGCGATGCTTACTG R: TGTAAATTGCCGCAAACGCC	Initial denaturation: 95°C, 5 min 35 cycles of: 1. Denaturation at 95°C for 30 s. 2. Annealing at 56°C for 30 s. 3. Extension at 72°C for 30 s. Final extension at 72°C for 5 min.	276 bp
rs340943904	*GBP5*	F: GACAGAAACGCTACCCATCGT R: CCTGCTGGTGCAGTCTGTTT	Initial denaturation: 95°C, 5 min 35 cycles of: 1. Denaturation at 95°C for 30 s. 2. Annealing at 55°C for 30 s. 3. Extension at 72°C for 30 s. Final extension at 72°C for 5 min.	402 bp

RESULTS AND DISCUSSION

In the 20 analyzed samples, the regions harboring the variants of interest were successfully amplified: rs80800372 in the GBP1 gene and rs340943904 in the GBP5 gene, resulting in fragments of 276 bp and 402 bp, respectively. Subsequently, from the sequencing of these fragments, the corresponding alleles for each SNP were identified. For SNP rs80800372 in *GBP1*, the allele frequencies were 0.825 for allele A and 0.175 for allele G. Regarding SNP rs340943904 in GBP5, the allelic frequencies were 0.825 for allele G and 0.175 for allele T. Both variants were found to be in Hardy–Weinberg equilibrium in the studied population sample (P>0.4678 in both cases). When considering only Pampa Rocha breed animals (N=14), there was an increase in the frequency of favorable alleles (G for rs80800372 and T for rs340943904), both rising from 0.175 to 0.215. For SNP rs80800372, the expected genotypic frequencies were 0.68 for AA, 0.28 for AG, and 0.03 for GG. These values were repeated for genotypes GG, GT and TT, respectively, in rs340943904. The Fis values for each locus were 0.159. FIG. 1 shows the alignments generated using the Bioedit program [24] for both variants.

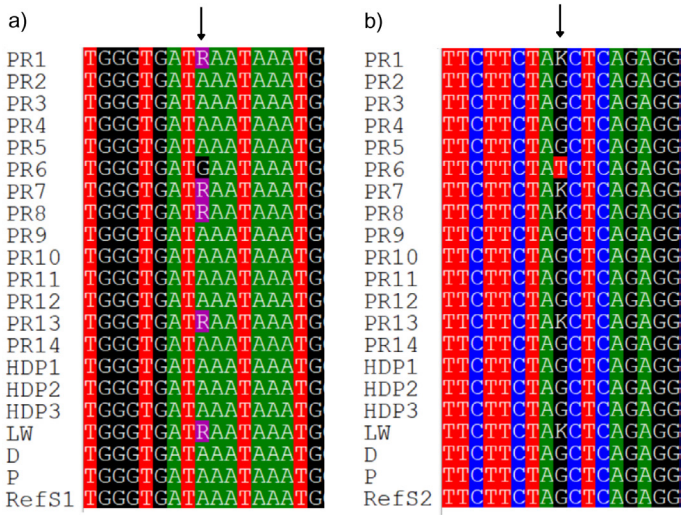

FIGURE 1. Alignments of the analyzed sequences in the GBP1 (a) and GBP5 (b) genes. Each sample is identified on the left (PR: Pampa Rocha, HDP: Pampa Rocha–Duroc hybrids, LW: Large White, D: Duroc, P: Pietrain, RefSeq-*GBP1*: Reference sequence of *GBP1*, RefSeq-*GBP5*: Reference sequence of *GBP5*). The arrows indicate variants rs80800372 (a) and rs340943904 (b). The letters A, G and T indicate that the animal's genotype is homozygous AA, homozygous GG, and homozygous TT, respectively. The letter R indicates genotypes AG and the letter K indicates genotypes GT.

Various studies have demonstrated that certain pig breeds exhibit greater resistance to PRRS. Notably, Chinese breeds such as Tongcheng and Meishan are known for their elevated resistance to PRRSV [30, 4]. Additionally, other native Chinese breeds and Tibetan pigs show differential susceptibility to PRRS infections [31, 32]. In the case of commercial breeds, it has been observed that Duroc females generally exhibit greater resilience to PRRS than Landrace sows [33]. To verify the trend observed in this study in Pampa Rocha pigs, the number of individuals studied should be increased. It is worth noting that genetic studies in the Pampa Rocha breed have indicated the influence of Asian breeds in their origin [34].

Regarding the linkage disequilibrium test, a correlation coefficient of 0.99 was determined, indicating a significant correlation between both variants in the analyzed pig sample. This is due to their proximity on chromosome 4 and explains the similarity in allelic and genotypic frequencies, as certain allele combinations occur more frequently. The presence of strong linkage disequilibrium between these SNPs on SSC4 has been reported in hybrid pigs [14, 35] and Yorkshire [12], among others. However, Kim *et al.* [36] did not find elevated correlations in Korean pig breeds. This correlation is useful for conducting genetic association studies, using one of the SNPs as a marker to identify nearby variants associated with a specific trait, such as the causative mutation in *GBP5*. The rs80800372 SNP (WUR), which is in linkage disequilibrium with the putative causative mutation in *GBP5*, can serve as a genetic marker for studying this mutation, as it is not present in commercial genotyping platforms [13]. *GBP5* has been identified by Koltes *et al.* [12] as a strong candidate gene for PRRS resistance/susceptibility. This conclusion is based on its differential expression during PRRSV infection, the presence of splice variant differences among animals with different genotypes, and its role in inflammasome assembly during the immune response.

In addition to the polymorphisms studied in this work, it would be of interest to analyze other markers in *GBP1* and *GBP5*, as well as in other genes such as *GBP2*, *GBP4*, *GBP6*, *CCBL2*, *GTF2B*, *PKN2*, and *CD163*, as associations with PRRS infection resistance in pigs have been reported [9, 35]. It is also important to explore other factors collectively, such as viral load and weight variations, as host resistance to PRRSV is estimated through a combination of these factors [9]. Furthermore, studying gene expression would provide a more comprehensive understanding of the mechanisms underlying resistance to this disease.

Controlling PRRS presents a complex challenge that requires a combination of diverse measures, given the virus's high genetic variability and the limited efficacy of current vaccines [3, 5]. It is crucial to explore alternative approaches for its management. One such approach involves identifying resistant individuals through genotyping of candidate genes across different pig breeds. It is essential to consider local zoogenetic resources, as these animals are often better adapted to local production systems, exhibiting greater hardiness and reduced selection pressure. In the case of the Pampa Rocha pig, due to the findings of this study and the influence of Asian breeds in its origin (which tend to be more resistant to PRRSV infections), further research is warranted.

Currently, leveraging the availability of cost-effective genomic information and advanced genetic selection tools offers opportunities to enhance resistance and monitor detrimental variants. However, biosecurity, disease surveillance and vaccination remain essential. An integrated approach across disciplines is essential for effectively preventing, controlling, and eradicating diseases like PRRS [7].

Improving understanding of animal resistance to diseases such as PRRS not only benefits production and animal welfare but also promotes the sustainability of pig farming. This is particularly crucial when considering local zoogenetic resources and the producers working with them.

CONCLUSIONS

The results of this study demonstrate the presence of genetic variants in the *GBP1* and *GBP5* genes that may be implicated in PRRSV resistance in Pampa Rocha pigs from Uruguay. An increase in the frequency of favorable alleles was observed in this population, along with strong linkage disequilibrium among the studied SNPs. These findings suggest the importance of continuing research on these candidate genes, as well as exploring other genes and factors related to disease resistance. Confirming the trend found in this work would further enhance the value of the local Pampa Rocha pig breed. The use of DNA molecular markers for identifying resistant animals could be a valuable tool for improving pig production and animal welfare, with a focus on utilizing local zoogenetic resources.

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Conflict of interest statement

The authors declare no conflict of interest.

BIBLIOGRAPHIC REFERENCES

[1] Abella G, Pena RN, Nogareda C, Armengol R, Vidal A, Moradell L, Tarancon V, Novell E, Estany J, Fraile L. A WUR SNP is associated with European Porcine Reproductive and Respiratory Virus Syndrome resistance and growth performance in pigs. Res. Vet. Sci. [Internet]. 2016; 104:117–122. doi: https://doi.org/f8bw9v

[2] You X, Li G, Lei Y, Xu Z, Zhang P, Yang Y. Role of genetic factors in different swine breeds exhibiting varying levels of resistance/susceptibility to PRRSV. Virus Res. [Internet]. 2023; 326:199057. doi: https://doi.org/gs642r

[3] Guo C, Liu X. Editorial: Porcine reproductive and respiratory syndrome virus – animal virology, immunology, and pathogenesis. Front. Immunol. [Internet]. 2023; 14:1194386. doi: https://doi.org/gtn97d

[4] Pei Y, Lin C, Li H, Feng Z. Genetic background influences pig responses to porcine reproductive and respiratory syndrome virus. Front. Vet. Sci. [Internet]. 2023; 10:1289570. doi: https://doi.org/gs65df

[5] Pileri E, Mateu E. Review on the transmission porcine reproductive and respiratory syndrome virus between pigs and farms and impact on vaccination. Vet. Res. [Internet]. 2016; 47:108. doi: https://doi.org/f89dr6

[6] Pena RN, Fernández C, Blasco-Felip M, Fraile LJ, Estany J. Genetic Markers Associated with Field PRRSV-Induced Abortion Rates. Viruses. [Internet]. 2019; 11(8):706. doi: https://doi.org/m7wb

[7] Harlizius B, Mathur P, Knol EF. Breeding for resilience: new opportunities in a modern pig breeding program. J. Anim. Sci. [Internet]. 2020; 98(Suppl. 1):S150–S154. doi: https://doi.org/m7wc

[8] Dekkers J, Rowland RRR, Lunney JK, Plastow G. Host genetics of response to porcine reproductive and respiratory syndrome in nursery pigs. Vet. Microbiol. [Internet]. 2017; 209:107–113. doi: https://doi.org/gchvxw

[9] Wu Q, Han Y, Wu X, Wang Y, Su Q, Shen Y, Guan K, Michal JJ, Jiang Z, Liu B, Zhou X. Integrated time-series transcriptomic and metabolomic analyses reveal different inflammatory and adaptive immune responses contributing to host resistance to PRRSV. Front. Immunol. [Internet]. 2022; 13:960709. doi: https://doi.org/gsgtc6

[10] Boddicker N, Waide EH, Rowland RRR, Lunney JK, Garrick DJ, Reecy JM, Dekkers JCM. Evidence for a major QTL associated with host response to porcine reproductive and respiratory syndrome virus challenge. J. Anim. Sci. [Internet]. 2012; 90(6):1733–1746. doi: https://doi.org/fzww9v

[11] Boddicker NJ, Garrick DJ, Rowland RRR, Lunney JK, Reecy JM, Dekkers JCM. Validation and further characterization of a major quantitative trait locus associated with host response to experimental infection with porcine reproductive and respiratory syndrome virus. Anim. Genet. [Internet]. 2014; 45(1):48–58. doi: https://doi.org/f5nb77

[12] Koltes JE, Fritz-Waters E, Eisley CJ, Choi I, Bao H, Kommadath A, Serão NVL, Boddicker NJ, Abrams SM, Schroyen M, Loyd H, Tuggle CK, Plastow GS, Guan L, Stothard P, Lunney JK, Liu P, Carpenter S, Rowland RRR, Dekkers JCM, Reecy JM. Identification of a putative quantitative trait nucleotide in guanylate binding protein 5 for host response to PRRS virus infection. BMC Genomics. [Internet]. 2015; 16:412. doi: https://doi.org/f7nkhw

[13] Dunkelberger JR, Serão NVL, Weng Z, Waide EH, Niederwerder MC, Kerrigan MA, Lunney JK, Rowland RRR, Dekkers JCM. Genomic regions associated with host response to porcine reproductive and respiratory syndrome vaccination and co-infection in nursery pigs. BMC Genomics. [Internet]. 2017; 18:865. doi: https://doi.org/m7wd

[14] Jeon RL, Cheng J, Putz AM, Dong Q, Harding JCS, Dyck MK, Plastow GS, Fortin F, Lunney J, Rowland R, *et al.* Effect of a genetic marker for the GBP5 gene on resilience to a polymicrobial natural disease challenge in pigs. Livest Sci. [Internet]. 2021; 244 (Suppl. 4):104399–101413. doi: https://doi.org/m7wf

[15] Lunney JK, Benfield DA, Rowland RRR. Porcine reproductive and respiratory syndrome virus: an update on an emerging and re-emerging viral disease of swine. Virus Res. [Internet]. 2010; 154(1–2):1–6. doi: https://doi.org/ftw8kf

[16] Ramos N, Betancour G, Puig J, Arbiza J. An update on genetic analysis of porcine reproductive and respiratory syndrome virus type 2 (PRRSV-2) in South America: identification of ORF5 sequences of lineage 1A, 1C and 1G. Arch. Microbiol. [Internet]. 2022; 204(7):367. doi: https://doi.org/m7wg

[17] Ramos N, Mirazo S, Castro G, Cabrera K, Osorio F, Arbiza J. First-time detection of porcine reproductive and respiratory syndrome virus (PRRSV) infection in Uruguay. Transbound. Emerg. Dis. [Internet]. 2018; 65(2):352–356. doi: https://doi.org/gcvjqg

[18] Castro G. Situación de los recursos genéticos porcinos locales en Uruguay. Arch. Zootec. [Internet]. 2007 [cited 6 Feb 2024]; 56(Supl.1):783–788. Available in: https://goo.su/ww0FN

[19] Food and Agriculture Organization of the United Nations (FAO). Plan de acción mundial sobre los recursos zoogenéticos y la Declaración de Interlaken [Internet]. Roma: FAO; 2007 [cited February 6, 2024]. 52 p. Disponible en: https://goo.su/zkeqY

[20] Barlocco N. Experiencias en la caracterización productiva del cerdo Pampa Rocha en Uruguay. Producción de carne natural. En: Llambí S, coordinadora. Situación y conservación de recursos zoogenéticos porcinos. Montevideo (UY): Oficina de Publicaciones de Facultad de Veterinaria (Udelar); 2011. p. 31–39.

[21] Vadell A, Barlocco N, Carballo C. Prolificidad y longevidad productiva de cerdas Pampa Rocha en un sistema de producción al aire libre. Rev. Computadorizada Prod. Porc. [Internet]. 2010 [cited 14 Feb 2024]; 17(2):149–153. Disponible en: https://goo.su/040z0

[22] Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA. [Internet]. 1977; 74(12):5463–5467. doi: https://doi.org/dgsrk5

[23] Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinform. [Internet]. 2012; 13:134. doi: https://doi.org/f38qjk

[24] Hall TA. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 1999; 41:95–98.

[25] Weir BS, Cockerham CC. Estimating F-Statistics for the analysis of population structure. Evolution. [Internet]. 1984; 38(6):1358–1370. doi: https://doi.org/gg27rg

[26] Belkhir K, Borsa P, Chikhi L, Rafauste N, Bonhomme F. Page WEB de GENETIX [homepage on the Internet]. Montpellier (FR): Université de Montpellier II, Institut des Sciences de l'Evolution; 2004 [cited 14 Feb. 2024]. Available in: https://goo.su/DbgsF

[27] Raymond M, Rousset F. GENEPOP (version 1.2): Population Genetics Software for Exact Tests dnd Ecumenicism. J. Heredity [Internet]. 1995; 86(3):248–249. doi: https://doi.org/gfkmsg

[28] Rousset F. 2008. GENEPOP'007: A Complete Reimplementation of the Genepop Software for Windows and Linux. Mol. Ecol. Resour. [Internet]. 2008; 8(1):103–106. doi: https://doi.org/c38k86

[29] Black WC, Krafsur ES. A FORTRAN Program for the Calculation and Analysis of Two-locus Linkage Disequilibrium Coefficients. Theor. Appl. Genet. [Internet]. 1985; 70(5):491–496. doi: https://doi.org/dcmjrk

[30] Liang W, Li Z, Wang P, Fan P, Zhang Y, Zhang Q, Wang Y, Xu X, Liu B. Differences of immune responses between Tongcheng (Chinese local breed) and Large White pigs after artificial infection with highly pathogenic porcine reproductive and respiratory syndrome virus. Virus Res. [Internet]. 2016; 215:84–93. doi: https://doi.org/f8fkdw

[31] Meng C, Su L, Li Y, Zhu Q, Li J, Wang H, He Q, Wang C,Wang W, Cao S. Different Susceptibility to Porcine Reproductive and Respiratory Syndrome Virus Infection Among Chinese Native Pig Breeds. Arch. Virol. [Internet]. 2018; 163(8):2155–2164. doi: https://doi.org/gd3b6v

[32] Kang R, Ji G, Yang X, Lv X, Zhang Y, Ge M, Pan Y, Li Q, Wang H, Zeng F. Investigation on Host Susceptibility of Tibetan Pig to Infection of Porcine Reproductive and Respiratory Syndrome Virus Through Viral Challenge Study. Vet. Microbiol. [Internet]. 2016; 183:62–68. doi: https://doi.org/f79dx3

[33] Hickmann FMW, Braccini Neto J, Kramer LM, Huang Y, Gray KA, Dekkers JCM, Sanglard LP, Serão NVL. Host Genetics of Response to Porcine Reproductive and Respiratory Syndrome in Sows: Reproductive Performance. Front. Genet. [Internet]. 2021; 12:707870. doi: https://doi.org/m7wh

[34] Montenegro M, Llambí S, Castro G, Barlocco N, Vadell A, Landi V, Delgado JV, Martínez A. Genetic Characterization of Uruguayan Pampa Rocha Pigs with Microsatellite Markers. Genet. Mol. Biol. [Internet]. 2015; 38(1):48–54. doi: https://doi.org/f67n2g

[35] Khatun A, Nazki S, Jeong CG, Gu S, Mattoo SUS, Lee SI, Yang MS, Lim B, Kim KS, Kim B, Lee KT, Park CK, Lee SM, Kim WI. Effect of Polymorphisms in Porcine Guanylate-Binding Proteins on Host Resistance to PRRSV Infection in Experimentally Challenged Pigs. Vet. Res. [Internet]. 2020; 51:14. doi: https://doi.org/m7wj

[36] Kim S, Cho ES, Kim YS, Lim Y, Jeong SA, Song M, Lee KT, Kim JM. Novel Insight into Linkage Disequilibrium and Additive Effect of *GBP1* and *GBP5* SNP Haplotypes Associated with Porcine Reproductive and Respiratory Syndrome Virus Susceptibility in Korean Native Pigs. Anim. Genet. [Internet]. 2021; 52(6):897–898. doi: https://doi.org/gpw4nj