Genetic and Glycosylation Variability of the GP5 Glycoprotein in *Betaarterivirus americense* NADC34 Variant Isolates from Peru

Molecular Variability of the GP5 Glycoprotein in *Betaarterivirus americense*

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ABSTRACT

Betaarterivirus americense causes a clinical condition with a high epidemiological risk in Peru and worldwide. Therefore, the aim of this study was to identify the genetic diversity of circulating viruses in different commercial swine farms in Lima. A total of 24 viral sequences from our previously published work were analyzed, and phylogenetic inference was performed based on amino acid alignments. The results revealed that all isolates clustered within sublineage 1.5 of the representative NADC34 variant. Sequence analysis of ORF5 (GP5) identified a high number of amino acid substitutions in key regions, including aa34 (24/24), aa39 (6/24), aa44 (4/24), aa47 (24/24), aa57 (24/24), aa58 (24/24), and aa59 (24/24), located within epitopes A, B, and C.

N-glycosylation analysis revealed 10 potential N-glycosylation sites: N30, N32, N33, N34, N35, N44, N51, N57, N128, and N151. Among these, the major conserved sites (N34, N44, and N51) were fully preserved in strains 40 and 41; in 20 out of 24 strains, only sites N44 and N51 were conserved, whereas strains 36, 37, 38, and 39 showed complete loss of these glycosylation sites. The signal peptide cleavage site was identified between amino acids 1 and 31.

Genetic diversity analysis revealed rapid viral evolution and a high mutation rate, with 530 variable sites and 860 mutations. Tajima's D statistical analysis indicated population subdivision, yielding a D

value of −0.78746. The high number of substitutions in key domains of the GP5 glycoprotein and the variability of potential N-glycosylation sites among the 24 NADC34 variant isolates of *Betaarterivirus americense* suggest that these strains exhibit high genetic variability and recombination potential.

Keywords: *Betaarterivirus americense*, glycoprotein 5, N-glycosylation, NADC34

INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is a viral disease of major sanitary importance that poses a serious threat to the global swine industry [13]. The etiological agent, porcine reproductive and respiratory syndrome virus (PRRSV), has been molecularly and structurally characterized, and two main species have been identified: *Betaarterivirus americense* and *Betaarterivirus europensis* [3,5,6].

The PRRSV genome is a positive-sense single-stranded RNA that contains a 5′ cap structure and a 3′ polyadenylated tail. It includes a 5′ untranslated region (UTR), at least ten open reading frames (ORFs)—ORF1a, ORF1b, ORF2a, ORF2b, ORF3, ORF4, ORF5, ORF5a, ORF6, and ORF7—and a 3′ UTR [27,31]. ORF1a and ORF1b comprise approximately three-quarters of the viral genome and encode the replicase polyproteins pp1a and pp1a/b, which undergo extensive proteolytic processing by virus-encoded proteases to produce at least 16 nonstructural proteins (nsps) involved in viral replication and transcription [10,11]. The remaining ORFs located in the 3′ terminal region encode eight structural proteins, including four membrane-associated glycoproteins (GP2a, GP3, GP4, and GP5), three nonglycosylated membrane proteins (E, ORF5a, and M), and one nucleocapsid protein (N) [8,32].

Phylogenetic analysis of the ORF5 gene in *Betaarterivirus americense* has identified nine monophyletic lineages (L1–L9), as well as three Korea-specific lineages (KOR A, B, and C) and several sublineages [6,20]. This pronounced genetic diversity, attributed to the high heterogeneity and variability of glycoprotein 5 (GP5), supports the hypothesis that PRRSV variants may inherently acquire distinct virulence patterns and clinical presentations.

The ORF5 gene encodes the major viral envelope glycoprotein (GP5), a transmembrane glycosylated protein exposed on the virion surface that plays a critical role in virus attachment to host cell receptors and represents a key target of the humoral immune response [1,7,25]. GP5 consists of a signal peptide, two hypervariable regions (HVR1 and HVR2), three transmembrane domains (TM1, TM2, and TM3), a decoy epitope, the primary neutralizing epitope (PNE), an additional B-cell epitope, and two T-cell epitopes [35]. This protein is essential for viral infectivity and contains important immunological domains associated with viral neutralization, including antigenic determinants and N-glycosylation sites [18].

GP5 glycosylation is an important immune evasion mechanism used by several enveloped viruses to escape, block, or minimize the activity of neutralizing antibodies [2]. In particular, changes in N-glycosylation sites associated with the primary neutralizing epitope significantly affect viral immunogenicity, rendering virions resistant to antibody neutralization in vivo [9]. In addition, recombination and mutation are the two main evolutionary mechanisms contributing to the emergence and evolution of new genetic variants of *Betaarterivirus americense*, playing a key role in the increasing number of circulating genotypes [14,15].

In Peru, following the molecular identification of lineage 1, sublineage 1.5, corresponding to the IA/2014/NADC34 variant in 2019 and 2025 [6,23], significant knowledge gaps remain regarding the prevalence of viral species and the genetic diversity of lineages associated with reduced immunogenicity, linked to the high genetic variability of *Betaarterivirus americense*. Therefore, the aim of this study was to identify the variability in the molecular architecture of glycoprotein 5 (GP5) in Peruvian isolates, contributing to a broader understanding of genetic variation, molecular epidemiology, immune evasion mechanisms, and the limitations in developing effective vaccines against PRRSV.

MATERIALS AND METHODS

Biological material

A total of 24 nucleotide sequences from strains isolated in the study by Cotaquispe [6] were analyzed in this investigation.

RNA extraction, quantification, and in-house conventional RT-PCR of the ORF5 gene

The procedures for obtaining viral cDNA of *Betaarterivirus americense* were carried out as described in the study by Cotaquispe [6].

Electrophoresis, purification, and sequencing

Amplified products were visualized using a CS Cleaver Scientific multiSUB™ Choice horizontal electrophoresis system with 1% molecular-grade agarose gels (SCL AG500, Cleaver Scientific), using Fluorescent Dye-DNA/Safe-Green™ (ABM®), a 100 bp Opti-DNA Marker (50 bp–1.5 kb, ABM®), and visualized with a UVP UV Solo Touch photodocumentation system (Analytik Jena). Gel fragments containing amplicons of the expected size were excised and purified using the innuPREP Nucleic Acid Purification Kit (Analytik Jena). Purified products were sequenced in both directions by Macrogen (South Korea) using the BigDye v3.1 Terminator Sequencing Kit (Life Technologies) and an ABI 3137 XL Genetic Analyzer (Life Technologies).

Phylogenetic analysis of glycoprotein 5 (GP5)

Sequenced chromatograms of glycoprotein 5 from this study were analyzed using Chromas Lite® v2.6.6 and combined with 58 reference sequences from GenBank. Nine of these corresponded to Peruvian isolates (MH791386.1; MH791385.1; MH791383.1; MH791388.1; MH791379.1; MH791378.1; MH791376.1; MH791391.1; MH791390.1), and the remaining sequences corresponded to pathogenic strains isolated worldwide (MF326985.1; DQ009647.1; DQ306879.1; DQ306878.1; DQ306877.1; GQ857656.1; FJ349261.1; AY588319.1; DQ489311.1; GQ461593.1; EF484033.1; AY032626.1; EF635006.1; KF611905.1; KF287140.1; JQ715697.1; EU939312.1; GQ374442.1; JQ663556.1; HQ315837.1; FJ889130.1; JX512910.2; JQ663545.1; JN411262.1; AY743931.1; JF802085.1; GU047345.1; EU076704.1; AY366525.1; KC862567.1; AF325691.1; KC862577.1; DQ473474.1; AB288356.1; JN660150.1; EU807840.1; EU262603.1; AF184212.1; DQ176019.1; AF331831.1; AF176348.2; U87392.3; MF279253.1; EF536003.1; AF066183.4; DQ988080.1; AY424271.1; AF494042.1; EF112445.1).

The phylogenetic tree was inferred based on amino acid substitution analysis using the maximum likelihood method with BioNJ and the Jones–Taylor–Thornton (JTT) model. The tree was drawn to scale, with branch lengths representing the number of substitutions per site. A total of 201 amino acids were included in the final dataset. Bootstrap values were calculated using 1,000 alignment replicates. Evolutionary analyses were performed using DNAMAN 10 and MEGA 6 [28].

Analysis of immunological domains of glycoprotein 5

Amino acid analysis was performed using MEGA 6.0 to determine the viral structure, including the signal peptide, decoy epitopes I and II, hypervariable regions I and II, the primary neutralizing epitope (PNE), transmembrane regions 1, 2, and 3, T-cell recognition epitopes 1 and 2, and the B-cell epitope. This analysis allowed identification of genetic variants, as mutations in viral protein structures may affect infectivity, pathogenicity, and viral persistence [17]. GP5 glycoprotein length was determined, amino acid mutations in key immunological domains were identified, and putative N-glycosylation sites were predicted using the NetNGlyc 1.0 server [12]. In addition, signal peptides were predicted using SignalP 4.0 [22].

Genetic diversity analysis: Tajima's D test and DnaSP v6

Statistical analysis was based on the neutral theory of molecular evolution, which assumes that the evolution of slightly deleterious or beneficial, nearly neutral mutations depends primarily on the mutation rate, selection coefficients, and population size. Tajima's D statistic [29] compares two estimates of genetic variation (θ) within a group of sequences. D is based on the difference between these estimates and has a mean of zero with a deviation of ± 1 ($-1 < D < +1$). The D value was compared with Table 2 [30] according to the number of samples and the closest confidence limit to the obtained D value. Negative deviations indicate non-neutrality, whereas positive D values may indicate positive

selection or a reduction in population size. The DnaSP v6 program was used to calculate Tajima's D values and to identify the genetic diversity of our isolates [24].

RESULTS

Serum samples were analyzed using an in-house kit with primers targeting the ORF5 gene, which encodes glycoprotein 5 (GP5) of the virus. The primers were validated and standardized using vaccine strains from both species as positive controls and a commercial IDEXX positive control mixture (Figure 1). Amplification of the gene yielded a product of 723 bp (Figure 2). Sequencing of all partial amplicons contained the genes encoding glycoprotein 5 (GP5), with lengths of 603 nucleotides and 201 functional amino acids.

Phylogenetic analysis of glycoprotein 5 (GP5)

The phylogenetic relationship of the study isolates, based on amino acid sequence alignment using representative GenBank strains from lineages 1, 5, and 8, is shown in Figure 3. In addition, nine isolates reported as Peruvian belonging to the sublineage 1.5 (1-7-4) variant of NADC34 were included. A 100% support value was observed in the generated tree for the presence of the species *Betaarterivirus europense* and *Betaarterivirus americense*, as well as multiple branches corresponding to groups formed by representative variants of species 2 from Lineage 1: sublineage 1.1 USA (99-3584, 99-3298, and 98-3403), sublineage 1.5 NADC34, sublineage 1.6 (NADC31), sublineage 1.8 (HENAN), sublineage 1.9 (MN184A); from Lineage 5: sublineage 5.1 (RespPRRS_MLV and VR2332); and from Lineage 8: sublineage 8.9 (Ingelvac_ATP) and sublineage 8.7 (JXA1). Representative variants of species 1 from Lineage 1-sublineage 1.A South Korea (SNU1 and KNU-07), sublineage 1.C (EuroPRRSV, CRESA-VP21, and Porcilis GP5) and from Lineage 3 (BELGA-Lena) were also included. Genealogy showed that the study isolates (turquoise branch) clustered with Peruvian isolates reported in 2015, 2016, and 2017 and demonstrated a close relationship with the highly pathogenic Lineage 1, sublineage 1.5 NADC34 variant first described in North America (Figure 3). Likewise, high divergence of strains 36, 37, 38, 39, and 42 was observed within sublineage 1.5.

Amino acid analysis of GP5

The obtained sequences were analyzed to predict potential N-glycosylation sites within glycoprotein 5 (GP5), as these are involved in viral immune evasion by minimizing the neutralizing capacity of epitopes. Glycosylation sites N33, N44, and N51 are located within the main neutralizing epitopes. The results demonstrated the presence of 10 potential N-glycosylation sites (N30, N32, N33, N34, N35, N44, N51, N57, N128, and N151). Of these, 20/24 isolates presented sites N44 and N51 within the primary neutralizing epitope (PNE). In addition, all isolates exhibited three or more N-glycosylation sites in their sequences. Notably, new sites not reported in reference strains (N57, N128,

and N151) were identified, and a higher number of sites was observed in strains 40 and 41 (Table 1). Furthermore, signal peptide cleavage site prediction analysis was performed to determine the site of enzymatic cleavage; the peptidase cleavage occurs within residues 1–31 of the protein sequence (Table 1).

Genetic diversity of glycoprotein 5 (GP5)

The structure of the translated amino acid sequences (201 aa) consisted of a signal peptide (aa 1–31), decoy epitope I (A) (aa 27–36) and decoy epitope II (aa 180–197), specialized in immune response neutralization, as well as the primary neutralizing epitope (B) (aa 37–45), neutralizing epitope (C) (aa 52–61), transmembrane region 1 (aa 65–82), transmembrane region 2 (aa 96–102), and transmembrane region 3 (aa 110–127), T-cell recognition epitope 1 (aa 117–131), T-cell recognition epitope 2 (aa 150–163), B-cell epitope (aa 180–200), and the stop codon (aa 201) (Figure 4).

Multiple amino acid substitutions were identified in the antigenic regions of the synthesized glycoprotein. Specifically, several neutral substitutions were observed in the 27 aa corresponding to the signal peptide, as well as nonsynonymous substitutions with important functional implications in the early antibody response mediated by decoy epitope I (A), particularly in hypervariable region 1. These substitutions included 15/24 at S32N/G/R/E, 24/24 at D34N/S/T, and 5/24 at S35N/I, which may induce rapid and potent non-neutralizing antibodies. Decoy epitope II also showed important amino acid substitutions (24/24 at P185A, 24/24 at L189V, 2/24 at T190P, and 24/24 at R191K), which play an important role in cross-neutralization responses to antibodies generated against species 1 and 2.

In addition, regions responsible for the production of neutralizing antibodies were analyzed. Neutralizing epitope B (PNE) is characterized by a highly conserved region comprising antigenic determinant sites (aa 39, 41, and 44). Our results showed substitutions in 7/24 isolates at aa L39F, 6/24 at aa Q40L/R, 3/24 at aa L41Y/V, 4/24 at aa N44I, and 24/24 at aa L47I/V. Neutralizing epitope C, together with its hypervariable region 2, includes antigenic determinants at positions aa 57, 58, 59, and 61, where substitutions enable escape from neutralizing antibodies. Substitutions were observed in 24/24 isolates at A57N, 24/24 at N58K/E/V/R, and 24/24 at N59H/S/R/N. Amino acid changes in epitopes A, B, and C of GP5 in field strains may reduce the protective effect of commercial PRRS vaccines.

Statistical analysis: Tajima's D test and genetic diversity using DnaSP v6

Genetic diversity analysis using the DnaSP program revealed 860 mutations, 530 polymorphic sites, and 61 conserved sites across the sequences. In addition, Tajima's D test yielded a value of −0.78746, which was not statistically significant ($P > 0.10$). This negative value indicates that the hypothesis of neutral evolution should not be rejected and suggests the presence of recombination or a considerable

incidence of population subdivision with deleterious mutations. The applied genetic diversity analyses demonstrated viral variability and plasticity, and the results, compared with the Lineage 5 VR2332 strain, are presented in Table 2.

DISCUSSION

The results showed the identification of 24 isolates belonging to the species *Betaarterivirus americense*. Phylogenetic inference was performed using 58 sequences from major epidemic outbreaks worldwide retrieved from databases, revealing the presence of lineages 1, 5, and 8. In addition, the relationship observed between the 24 isolates and the Peruvian reference strains showed genetic divergence within Lineage 1, specifically sublineage 1.5 of the NADC34 variant, according to glycoprotein 5 studies and the methods described by Nguyen [19] and Shin [26]. Our data are consistent with those reported by Ramírez [23], who showed that Peruvian isolates, based on RFLP typing, are related to the 1-7-4 variant of Lineage 1, specifically sublineage 1.5 of NADC34.

Studies of the structural protein GP5 showed that antigenic domains play an important role in the immune response due to synonymous and nonsynonymous substitutions occurring in key immunological domains involved in the production of non-neutralizing antibodies, such as decoy epitopes I (A) and II, and neutralizing epitopes such as B and C, which contribute to immune evasion and neutralization [17,21]. In addition, the results demonstrated different degrees of substitution in other domains, including the signal peptide; transmembrane regions 1, 2, and 3; T-cell recognition epitopes (1 and 2); and the B-cell epitope, when compared with Lineage 5 of the VR2332 variant.

Our results showed 15/24, 24/24, and 5/24 substitutions at aa S32N/G/R/E, D34N/S/T, and S35N/I, respectively, in the hypervariable region 1 of the decoy epitope (A). The high variability of this region, according to Nguyen [19], induces rapid and strong non-neutralizing antibody responses. That is, the decoy epitopes aa27–35 and aa180–197 fulfill an immune evasion function: when pigs are infected with the virus, these two decoy epitopes first stimulate immune system cells to rapidly produce large amounts of non-neutralizing antibodies against these epitopes, thereby delaying the production of effective antibodies [35]. Furthermore, our findings of 7/24, 6/24, 3/24, 4/24, and 24/24 substitutions in amino acid regions L39F, Q40L/R, L41Y/V, N44I, and L47I/V of the primary neutralizing epitope (B) suggest high pathogenicity of our isolates, since substitutions in this region play an important role in immune neutralization [14]. These changes help PRRS field strains escape vaccine-induced neutralizing immunity, and mutation at aa39 has been used to classify viral variants according to Zhou [35]. In addition, studies by Faaberg [9] identified aa38, aa42, aa43, and aa44 as neutralizing activity sites, and aa39, aa40, and aa41 as antibody-binding sites. According to Chen [4], mutations in these regions can reduce the sensitivity of PRRSV-2 to neutralizing antibodies in vivo, thereby affecting

immune clearance of viral particles and potentially contributing to vaccination failure and increased morbidity in swine herds. Finally, our results showed 24/24 substitutions at A57N, 24/24 substitutions at N58K/E/V/R, and 24/24 substitutions at N59H/S/R/N in the neutralizing epitope C and its hypervariable region 2, where such substitutions enable escape from neutralizing antibodies [19]. Overall, these data demonstrate a high incidence of mutations located in key immunological domains within epitopes A, B, and C of GP5, which are responsible for the production of non-neutralizing and neutralizing antibodies in the field strains isolated in this study. Previous studies by Nguyen [19] showed that substitutions in these domains reduce the protective effect of commercial PRRS vaccines. This represents important information regarding the high pathogenicity of Peruvian isolates and the potential for recombination with vaccine strains if applied, which could lead to the emergence of new, more pathogenic variants in Peru.

Another major role of GP5 is the glycosylation of membrane proteins. This process involves the addition or removal of glycan residues at potential N-glycosylation sites in PRRSV species. The N34 site is more prone to mutation, whereas N44 and N51 are highly conserved, and mutations at N44 may lead to increased virulence [4]. Previous studies, such as those by Ansari [2], have shown that removal of N-glycosylation sites increases the susceptibility of PRRSV species to neutralization by specific immune antibodies. Therefore, an increased number of potential N-glycosylation sites may explain the continuous mutation of viral species. These statements are consistent with the findings of the present study, as the analysis revealed 10 potential N-glycosylation sites (N30, N32, N33, N34, N35, N44, N51, N57, N128, and N151) in the extravirion region of the glycoprotein in all 24 isolated strains.

The large number of potential N-glycosylation sites in the study strains was consistent with the results reported by Li [16], but contrasts with the findings of Xie [33] and Chen [4], who reported lower numbers of N-glycosylation sites. However, in this study, strains 36, 37, 38, 39, 43, and 44 exhibited three N-glycosylation sites, whereas strains 40 and 41 showed six N-glycosylation sites with greater observable variability. According to Ansari [2], the three conserved potential glycosylation sites (N34, N44, and N51) contribute to viral variability and failure of control by commercial vaccines, and they indicate that glycan addition at site N44 is particularly critical because it enhances viral escape from antibodies. Furthermore, these authors argue that PRRSV species containing N-hypoglycosylated forms of GP5 are sensitive to antibody neutralization, and that removal of glycosylation sites in laboratory-generated mutant viruses induces significantly higher levels of neutralizing antibodies by the immune system.

The data obtained in this study suggest that hypoglycosylated wild-type viruses are less common than highly glycosylated strains, which may explain the expansion of viral variants. The effect of N-glycosylation suggests that glycans mask one or more neutralizing epitopes, resulting in the failure to induce neutralizing antibodies when glycans are present [2]. In addition, only strains 40 and 41 retained

all three glycosylation sites (N34, N44, and N51), whereas strains 36, 37, 38, and 39 lost them. Moreover, conserved potential N-glycosylation sites N44 and N51 were present in 20 of the 24 analyzed isolates, and 18 of the 24 had more than three N-glycosylation sites. According to Zhang [34], a higher number of N-glycosylation sites may contribute to reduced immunogenicity of nearby neutralizing epitopes, suggesting that these isolates may escape immune recognition and neutralization by vaccine-induced antibodies. This observation is highly relevant for the design of effective vaccines against emerging variants, suggesting that newly designed vaccines targeting these regions should incorporate modifications in the glycosylation pattern of the GP5 gene to enhance the production of neutralizing antibodies [2]. Nevertheless, further studies are required to determine whether mutation of glycosylation sites affects exposure of viral neutralizing epitopes, as well as immune evasion and viral pathogenicity [4].

Genetic diversity analysis of the 24 strains using the DnaSP program showed rapid evolution and a high mutation rate in Peruvian isolates, with 530 variable sites and 860 mutations. In addition, Tajima's D test revealed a considerable incidence of population subdivision, yielding a D value of −0.78746 [24]. Overall, our results reflect the reality of rapid evolution and spread of PRRSV variants in Peru and help explain the ongoing problem of vaccine inefficacy due to immune evasion mechanisms associated with increased N-glycosylation sites in Peruvian variants.

CONCLUSIONS

- Through phylogenetic analysis, we identified the relationship between the 24 study isolates and the Peruvian reference strains with Lineage 1, sublineage 1.5 of the NADC34 variant, based on glycoprotein 5 alignment.
- We identified a high number of significant substitutions in immunological domains involved in the production of non-neutralizing and neutralizing antibodies: aa34 (24/24), aa39 (6/24), aa44 (4/24), aa47 (24/24), aa57 (24/24), aa58 (24/24), and aa59 (24/24), located in epitopes A, B, and C of GP5.
- We identified 10 potential N-glycosylation sites (N30, N32, N33, N34, N35, N44, N51, N57, N128, and N151) in the 201–amino acid sequence of glycoprotein 5.
- We identified substitutions, losses, and conservation of N-glycosylation sites (N34, N44, and N51), with partial conservation in most strains or complete conservation in strains 40 and 41, and complete loss in strains 36, 37, 38, and 39.
- We identified that the 24 strains exhibit rapid evolution and a high mutation rate in Peruvian isolates, with 530 variable sites and 860 mutations.

- Using statistical analysis with Tajima's D test, we determined a considerable incidence of population subdivision, yielding a D value of −0.78746.

SUPPLEMENTARY MATERIAL

- Supplementary Material 1. Amino acid sequence alignment of glycoprotein 5 using MEGA 6.

CONFLICT OF INTEREST

- The authors declare that they have no conflicts of interest in submitting this article for publication.

FUNDING

- This work was carried out and funded at the facilities of the Animal Nutrition and Health Business Unit, Innovation and Development Area, Corporación Montana S.A.

ACKNOWLEDGMENTS

- The authors thank Montana S.A. for funding and support in conducting this research, and Bach. Segundo Del Águila Soto for logistical support and valuable suggestions.

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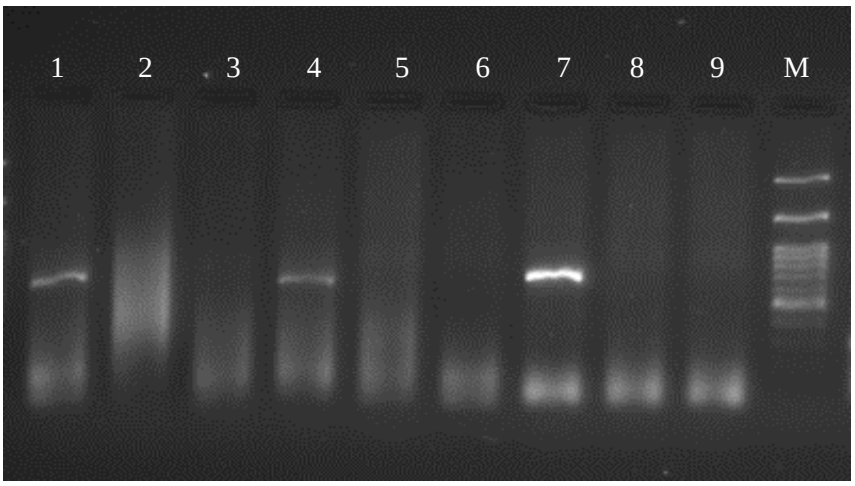

Figure 1. In-house conventional RT-PCR of the ORF5 gene designed in the laboratory. Lane 1 (ORF5-SKA): positive cDNA from the Nebraska Prime Pac® PRRS-2 strain; Lane 4 (ORF5-SKA): positive cDNA from the VP-046 SUIPRABAC® PRRS-1 strain; Lane 7 (ORF5-SKA): positive control cDNA mixture of PRRS-1 and PRRS-2 from the IDEXX commercial kit; Lanes 2, 5, and 8 (avian bronchitis): negative cDNA controls from IBV; Lanes 3, 6, and 9 (PCR blank): nuclease-free water; Lane M (ladder): 100 bp Plus Opti-DNA Marker ABM®. Image adapted from Cotaquispe et al., 2025.

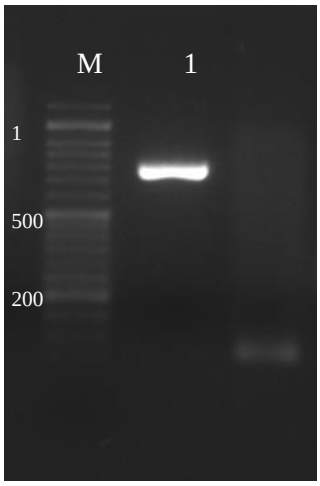

Figure 2. In-house conventional RT-PCR of the ORF5 gene designed in the laboratory. Lane 1 (ORF5-SKA): cDNA from a PRRS field-positive sample; Lane 2 (avian bronchitis): negative control cDNA from IBV; Lane M (ladder): 100 bp Plus Opti-DNA Marker ABM® (50 bp–1.5 kb).

Table 1. Results of the genotypic variability of 24 isolates of Porcine Reproductive and Respiratory Syndrome virus (PRRSV) from blood samples of pigs from the Lima region, Peru.

cDNA	GP5 (aa)	Genotyping	N-glycosylation								Signal peptide
			#	1	2	3	4	5	6		
25	201	Lineage 1: Sublineage 1.5	**5**	30	33	44	51	57			1-31
26	201	Lineage 1: Sublineage 1.5	**5**	30	33	44	51	57			1-31
27	201	Lineage 1: Sublineage 1.5	**5**	30	33	44	51	57			1-31
28	201	Lineage 1: Sublineage 1.5	**5**	30	33	44	51	57			1-31
29	201	Lineage 1: Sublineage 1.5	**4**	30	33	44	51				1-31
30	201	Lineage 1: Sublineage 1.5	**5**	32	35	44	51	57			1-31
31	201	Lineage 1: Sublineage 1.5	**5**	32	35	44	51	57			1-31
32	201	Lineage 1: Sublineage 1.5	**5**	32	35	44	51	57			1-31
33	201	Lineage 1: Sublineage 1.5	**5**	32	35	44	51	57			1-31
34	201	Lineage 1: Sublineage 1.5	**5**	30	33	44	51	57			1-31
35	201	Lineage 1: Sublineage 1.5	**5**	30	33	44	51	57			1-31
36	201	Lineage 1: Sublineage 1.5	**3**	33	50	128					1-31
37	201	Lineage 1: Sublineage 1.5	**3**	33	50	128					1-31
38	201	Lineage 1: Sublineage 1.5	**3**	33	50	128					1-31
39	201	Lineage 1: Sublineage 1.5	**3**	33	50	128					1-31
40	201	Lineage 1: Sublineage 1.5	**6**	30	33	34	44	51	57		1-31
41	201	Lineage 1: Sublineage 1.5	**6**	30	33	34	44	51	57		1-31
42	201	Lineage 1: Sublineage 1.5	**5**	33	44	51	57	151			1-31
43	201	Lineage 1: Sublineage 1.5	**3**	44	51	57					1-31
44	201	Lineage 1: Sublineage 1.5	**3**	44	51	57					1-31
45	201	Lineage 1: Sublineage 1.5	**4**	32	33	44	51				1-31
46	201	Lineage 1: Sublineage 1.5	**4**	32	33	44	51				1-31
47	201	Lineage 1: Sublineage 1.5	**4**	32	33	44	51				1-31
48	201	Lineage 1: Sublineage 1.5	**5**	32	33	44	51	57			1-31

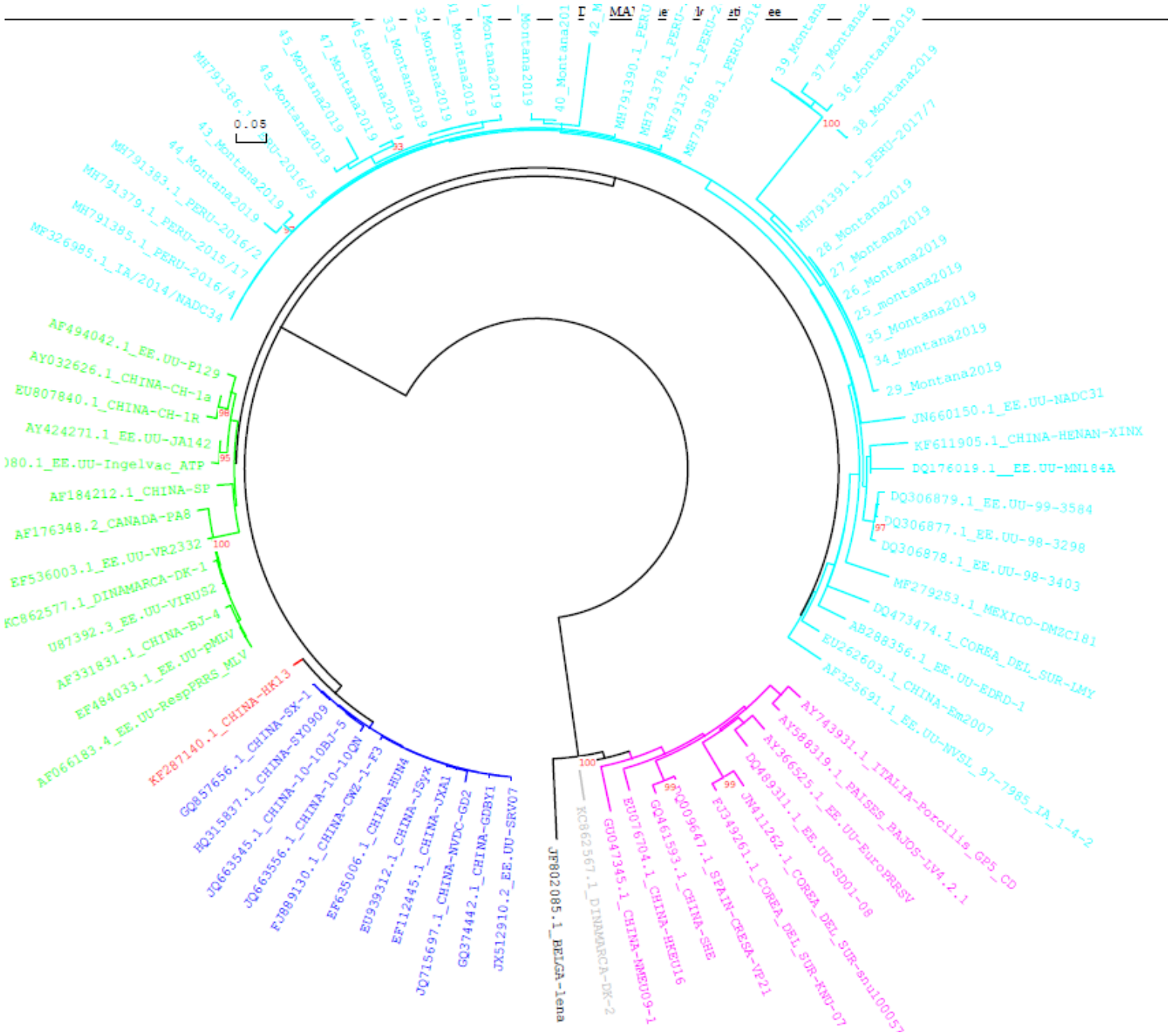
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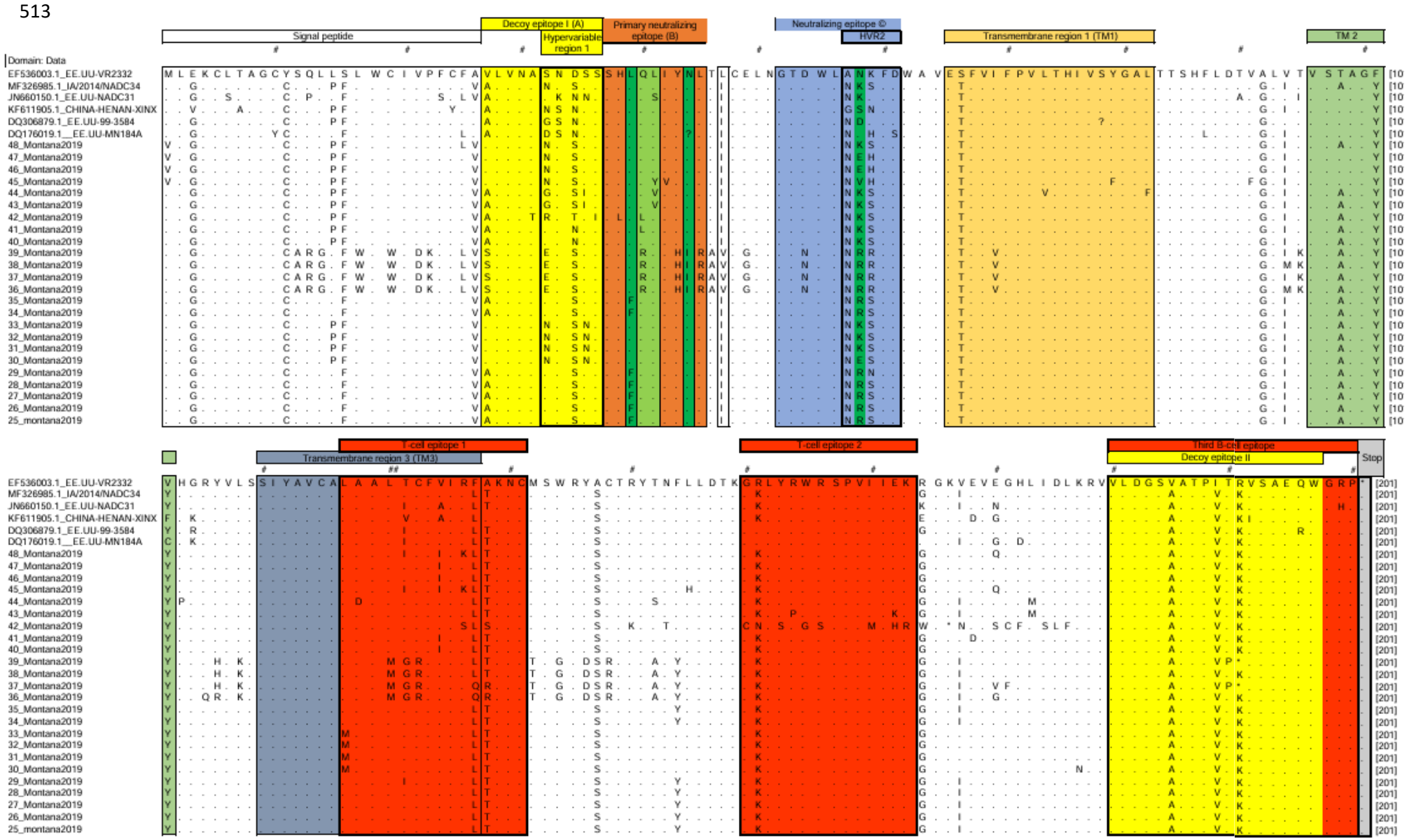
Figure 3. Phylogenetic tree of *Betaarterivirus europense* and *Betaarterivirus americense* species based on the glycoprotein 5 (GP5) sequence. (a) 1. Purple branching: *Betaarterivirus suid 1*; 2. Turquoise branching (Lineage 1): sublineage 1.1 (98-3584), sublineage 1.5 (NADC34, study isolates and Peruvian reference strains), sublineage 1.6 (NADC31), sublineage 1.8 (HENAN), sublineage 1.9 (MN184A); 3. Light green branching (Lineage 5): sublineage 5.1 (Res-PRRS MLV and VR2332); 4. Dark blue branching (Lineage 8): sublineage 8.7 (JXA1). (b) Amino acid substitution analysis was performed using the maximum likelihood method applying BioNJ with the Jones–Taylor–Thornton (JTT) model. Bootstrapping with 1,000 alignment replicates was used, and evolutionary analyses were conducted in MEGA6 and DNAMAN 10.

Table 2. Results of the genetic diversity of 24 isolates of Porcine Reproductive and Respiratory Syndrome virus (PRRSV) analyzed using the DnaSP and MEGA 6 programs from blood samples of pigs from the Lima region, Peru.

Strains		ORF 5 (GP5)	C.S.	Variable sites	P. I. S	U. V. S	Mutations
24	nt	603	61	530	48	82	860
	aa	201					

nt: nucleotide; **aa**: amino acid; **C.S.**: conserved sites; **P.I.S.**: parsimony-informative sites; **U.V.S**: unique variable sites.


Signal peptide
Decoy epitope I (A)
Hypervariable region 1
Primary neutralizing epitope (B)
Neutralizing epitope ©
HVR2
Transmembrane region 1 (TM1)
TM 2
Domain: Data
T-cell epitope 1
Transmembrane region 3 (TM3)
T-cell epitope 2
Third B-cell epitope
Decoy epitope II
Stop
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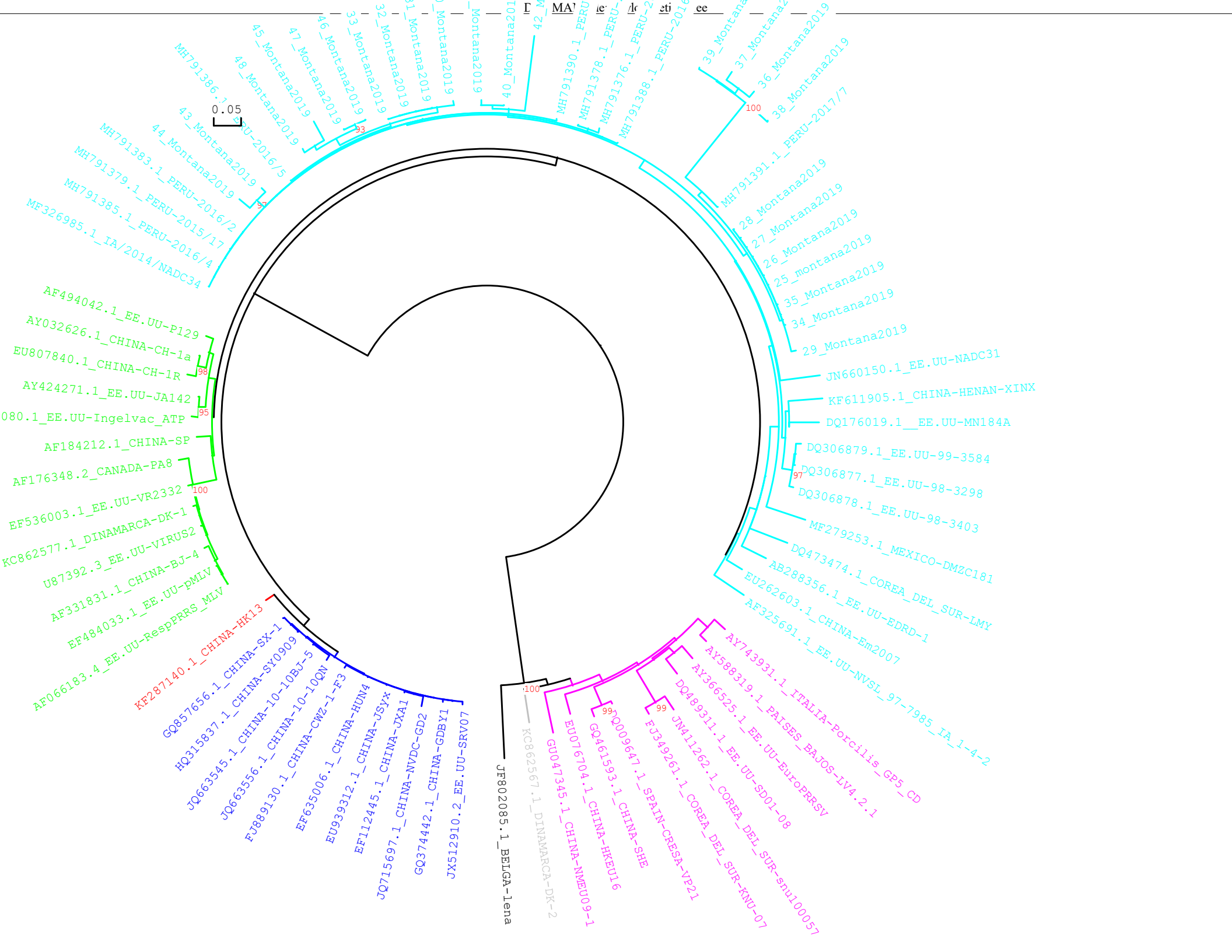
514 Figure 4. Alignment of amino acid sequences from antigenic regions in PRRSV GP5; the boxed and
515 colored regions represent antibody-neutralizing antigens. The 24 field isolates and
516 representative variants of sublineages 1.1 (99-3584), 1.5 (NADC34), 1.6 (NADC31), 1.8
517 (HENAN), 1.9 (MN184A), and sublineage 5.1 (VR2332) were used as reference patterns in
518 the alignment. Dots indicate the position of homologous amino acids, and letters indicate
519 amino acid substitutions. Yellow, orange, and blue boxes indicate the positions of non-
520 neutralizing and neutralizing epitope amino acids.

521

522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