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SHORT NOTE

Phylogenetic analysis of spike gene of porcine epidemic diarrhea virus in Colombia, 2014-2015

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Additional keywords

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SUMMARY

Outbreaks of porcine epidemic diarrhea were reported in Colombia during 2014-2015. In the present analysis, complete nucleotide sequences were downloaded from the Genbank for the Spike gene of porcine epidemic diarrhea virus (PEDV) strains reported in Colombia. In the phylogenetic tree based on spike proteins of PEDV, the strains from Colombia were clustered into genotypes G2a and S-INDEL, the strains showed high homology (> 99.9%) with strains from the United States (Colorado / 2013, OH851), as well as insertions and deletions in the S1 domain of the Spike gene present both in Colombian strains and reference strains. Several amino acid substitutions have been identified in the neutralizing epitopes for the Colombian strains compared to the CV777 reference strain. These studies could help to trace the possible routes of introduction of PEDV in Colombia, recognize the phylogenetic relationships of the strains circulating in the country, and implementing control and prevention strategies.

Análisis filogenético del gen Spike del virus de la diarrea epidémica porcina en Colombia, 2014-2015

Palabras clave adicionales

Colombia.
Virus de la diarrea epidémica porcina.
PEDV.
Análisis filogenético.
Gen spike.

RESUMEN

Se reportaron brotes de diarrea epidémica porcina en Colombia durante 2014-2015. En el presente análisis, se descargaron secuencias de nucleótidos completas del Genbank para el gen Spike de las cepas del virus de la diarrea epidémica porcina (PEDV) reportadas en Colombia. En el árbol filogenético basado en proteínas de espiga de PEDV, las cepas de Colombia se agruparon en los genotipos G2a y S-INDEL, las cepas mostraron alta homología (> 99,9%) con cepas de Estados Unidos (Colorado / 2013, OH851), como así como inserciones y deleciones en el dominio S1 del gen Spike presente tanto en cepas colombianas como en cepas de referencia. Se han identificado varias sustituciones de aminoácidos en los epítopos neutralizantes de las cepas colombianas en comparación con la cepa de referencia CV777. Estos estudios podrían ayudar a rastrear las posibles rutas de introducción del PEDV en Colombia, reconocer las relaciones filogenéticas de las cepas que circulan en el país e implementar estrategias de control y prevención.

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INTRODUCTION

Porcine epidemic diarrhea virus (PEDV) is a single-stranded positive-sense RNA virus, enveloped, member of the genus Alphacoronavirus in the family Coronaviridae, order Nidovirales. PEDV is the causative agent of porcine epidemic diarrhea (PED), an enteric disease affecting pigs of all ages, being more severe in neonatal piglets where mortality can reach 100%. Signs of PED are characterized by watery diarrhea, dehydration, and vomiting. The PEDV genome comprises 7 open reading frames (ORF1a, ORF1b and ORF2-6) encoding four structural proteins: spike protein (S), envelope (E), membrane (M), nucleocapsid (N). The spike protein contains two functional sub-units: S1, responsible for receptor cell binding and S2, responsible for membrane fusion (Jung & Saif 2015). PEDV first appeared in the United Kingdom in 1971 (Wood 1977). In 1977, a sudden outbreak of diarrhea occurred in pigs of all ages, within 4 pig farms in Belgium. Coronavirus-like particles were observed by electron microscopy and designated as CV777 strain (Pensaert & de Bouck 1978). Later, in the 1980s and 1990s, PEDV was identified as the cause of severe epidemics in Ja-

pan and South Korea (Takahashi, Okada & Ohshima 1983; Kweon et al. 1993).

Despite the use of PEDV vaccines, the disease has been endemic in South Korea (Park, Song & Park 2013). During the 1980s and 1990s in Europe, less frequent outbreaks of PED appeared, but the virus persisted in an endemic form in the swine population. Serologies showed a low to moderate prevalence of PEDV in European pigs (Van Reeth & Pensaert 1994). PED outbreaks were observed occasionally in Europe: in the Netherlands in 1989-1991 (Pijpers et al. 1993); in Hungary in 1995 (Nagy et al. 1996) and in England in 1998 (Pritchard et al. 1999). However, an epidemic of diarrhea caused by PEDV was reported in 63 herds in Italy during 2005–2006 (Martelli et al. 2008).

In October 2010, a highly pathogenic variant of PEDV generated a large-scale outbreak in China, with significant economic losses (Wang, Fang & Xiao 2016). PEDV first entered in the United States (US) in April 2013 (Stevenson et al. 2013), and this highly virulent strain spread rapidly through 36 states, as well as other countries in North and South America. The first year, PEDV caused the death of 10% of the pig population in the US, causing the death of 7 million pigs with significant economic losses for the North American country (Jung & Saif 2015). The last large-scale outbreak ended up in the spring of 2014 (Jarvis et al. 2016). The strains identified in the US were genetically related to Chinese strains (China / 2012 / AH2012) reported in 2011-2012 (Huang et al. 2013; Chen et al. 2014), indicating the emergence of Chinese PEDV strains (AH2012) in the US. In January 2014, a less virulent strain of PEDV (OH851) characterized by small genomic insertions and deletions (S-INDEL) in the viral spike gene was detected in the US (Wang, Byrum & Zhang 2014).

The virus spread to other countries in the Americas in 2014 including: Canada, Mexico, Peru, and Colombia (Vlasova et al. 2014a). In June 2014, Colombia, through the Instituto Colombiano Agropecuario (ICA), reported to the World Organization for Animal Health - OIE, the presence of 45 PEDV outbreaks in 5 departments of the country. From the emergency occurring in March 2014 due to the introduction of PEDV in Colombia, 162 cases were reported in 51 municipalities, of which 59 were presented in 2014, 98 in 2015 and in 2016, 5 cases. The most affected departments were Antioquia, Valle, Cundinamarca, Nariño, Huila, among others. The productive systems with major impacts were farrow- to- finish units with 58 farms, followed by breeding farms with 50 affected farms. The morbidity of PEDV in Colombia was higher in the population of suckling, weaned, and fattening pigs, with increased mortality rates in piglets (Fondo Nacional de Porcicultura 2016).

In Europe, PEDV re-emerged in 2014, being reported in the Netherlands (Dortmans et al. 2018), Austria (Steinrigl et al. 2015), Belgium (Theuns et al. 2015), Portugal (Mesquita et al. 2015) and France (Grasland et al. 2015). The reported strains are highly related to the strains found in Germany and S-INDEL (OH851) strains from the US (Hanke et al. 2015).

In October 2013, PEDV emerged in Japan, and 1,000 outbreaks were reported in 2015(Masuda et al. 2015). In Korea and Vietnam, the virus emerged in 2013, based on complete genomic sequences (Vui et al. 2014; Kim et al. 2015). In Thailand, highly virulent and classic strains were recently sequenced (Cheun-Arom et al. 2015). In 2014, outbreaks of PEDV occurred in Taiwan (Lin et al. 2014) and in the Philippines (Kim et al. 2016), phylogenetic studies with partial sequences of the viral S1 domain of the spike gene have been carried out in the Philippines (Paraguison-Alili & Domingo 2016) and Taiwan (Sung et al. 2019). Therefore, the aim of this work was the study of the genetic diversity and the phylogenetic relationship of the Spike gene of PEDV strains reported in Colombia during the outbreaks of 2014-2105.

MATERIALS AND METHODS

A phylogenetic tree based on spike proteins was constructed using the maximum likelihood algorithm, Jones Taylor Thornton model, and supported with a bootstrap test of 1000 replicates using Mega X software. The complete nucleotide sequences for the spike gene were downloaded from the Genbank database for global reference strains and for 18 Colombian PEDV strains reported in 2014-2015 from five departments of Colombia (Cundinamarca, Antioquia, Valle del Cauca, Cauca and Huila) using the keywords PEDV, Colombia and complete genome. Details about this prospective epidemiological study and the complete genome sequencing of these Colombian strains were described here (Qi et al, 2020). Nucleotide alignments and deduced amino acid alignments were performed by using the ClustalW method in MEGA X software and Bioedit (Hall 1999; Kumar et al. 2018). The matrix for the percentage of the nucleotide homology was done using geneious prime (www.geneious.com).

Genbank accession numbers for the Colombian strains: KU569509.1, MK071622.1, MK071623.1, K071624.1, MK071625.1, MK071626.1, MK071627.1, MK071628.1, MK071629.1, MK071630.1, MK071631.1, MK071632.1, MK071633.1, MK071634.1, MK071635.1, MK071636.1, MK071637.1, MK071639.1.

RESULTS AND DISCUSSION

The phylogenetic tree showed that the PEDV strains could be separated into two clusters: the classical genotype (G1) represented by the CV777 strain identified in Belgium, and the G2 genotype for pandemic strains. The G2 clade could be segmented into G2a, G2b, and S-INDEL genotypes. The Colombian strains clustered into G2a and S-INDEL genotypes (**Figure 1**). The S-INDEL strains refer to the strains that emerged after 2013 in the United States, and they are represented by the OH851 strain identified in January 2014, with lower infectivity compared to the non S- INDEL strains (Chen et al. 2019). Also, S-INDEL strains such as Minnesota52 and Indiana 12.38 / 2013 were found in samples collected in the US in June 2013, just a few months (1-2 months) after of the emergence of highly virulent PEDV strains in this country (Vlasova et al. 2014b). The nucleotide homology between the following Co-

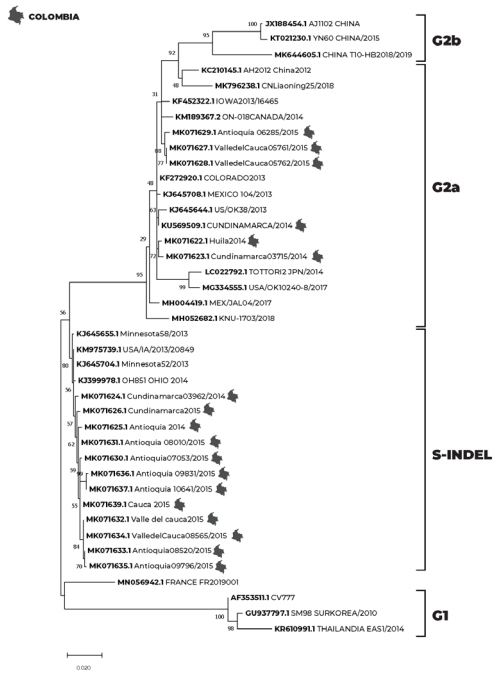

Figure 1. Phylogenetic tree based on spike proteins of global PEDV strains and 18 Colombian PEDV strains by maximum likelihood method using MEGA X with 1000 bootstrap replicates. The Colombian strains were marked with the Colombian map (Árbol filogenético basado en proteínas de espiga de cepas globales de PEDV y 18 cepas colombianas de PEDV por el método de máxima verosimilitud utilizando MEGA X con 1000 réplicas de arranque. Las cepas colombianas se marcaron con el mapa colombiano).

lombian strains: Cundinamarca2014, Huila2014, Cundinamarca03715/2014, ValledelCauca05761/2015, ValledelCauca05762/2015, Antioquia_06285/2015 and the Chinese strain (AH2012) and COLORADO2013 strain was 99.78-99.90 %.

For the following Colombian strains the similarity with the OH851/2014 strain from the US was very high, 99.64-99.90 % : Cundinamarca03962/2014, Antioquia2014, Cundinamarca2015, Antioquia07053/2015, Antioquia08010/2015, Valledelcauca2015, Antioquia08520/2015, ValledelCauca08565/2015, Antioquia09796/2015, Antioquia09831/2015, Antioquia_10641/2015, Cauca2015. Moreover, the nucleotide similarity between the G2a and S-INDEL genotypes for the Colombian strains was 95.73-97.80 %. The nucleotide similarity between all the Colombian strains and the CV777 strain is lower, being 93.64 – 95.76 % (**Table I**).

The deduced amino acid alignments showed some insertions and deletions in the S1 domain of the Spike gene, for Colombian strains compared to reference strains. There are 4-aa deletions at positions 59-62, 1-aa deletion at position 140, and 2-aa insertions at 159-160 in CV777 and OH851, as well as in the strains from Colombia: Cundinamarca03962_2014 , Antioquia_2014, Cundinamarca_2015, Antioquia07053, Antioquia 08010, ValledelCauca2015, Antioquia08520, Valledelcauca08565, Antioquia09796, Antioquia09831, Antioquia10641, Cauca2015. The opposite to the non S-INDEL strains such as AH2012, COLORADO2013, Cundinamarca 2014, Huila 2014, Cundinamarca03715/2014, ValledelCauca05761/2015, ValledelCauca05762/2015, Antioquia_06285/2015 with insertions of 4-aa at 59QGVN62, 1-aa insertion at 140N, and 2-aa deletions (159-160) (**Figure 2**).

Table I. Nucleotide homology between Colombian and reference PEDV strains (Homología de nucleótidos entre cepas de PEDV colombianas y de referencia).

Strain	CV777 (%)	AH2012 (%)	COLORADO 2013 (%)	OH851/2014 (%)
KU569509.1 Cundinamarca 2014	93,67	99,06	99,90	96,40
MK071622.1_Huila2014	93,64	99,04	99,88	96,38
MK071623.1_Cundinamarca03715/2014	93,74	99,04	99,88	96,38
MK071624.1_Cundinamarca03962/2014	95,74	95,61	96,45	99,90
MK071625.1_Antioquia_2014	95,64	95,51	96,35	99,81
MK071626.1_Cundinamarca2015	95,67	95,49	96,33	99,78
MK071627.1_ValledelCauca05761/2015	93,69	99,18	99,83	96,33
MK071628.1_ValledelCauca05762/2015	93,69	99,18	99,83	96,33
MK071629.1_Antioquia_06285/2015	93,71	99,14	99,78	96,30
MK071630.1_Antioquia07053/2015	95,76	95,54	96,38	99,83
MK071631.1_COL/Antioquia08010/2015	95,71	95,58	96,42	99,88
MK071632.1_Valledelcauca2015	95,74	95,51	96,35	99,81
MK071633.1_Antioquia08520/2015	95,71	95,49	96,33	99,78
MK071634.1_ValledelCauca08565/2015	95,67	95,46	96,30	99,76
MK071635.1_Antioquia09796/2015	95,67	95,44	96,28	99,74
MK071636.1_COL/Antioquia09831/2015	95,52	95,42	96,23	99,64
MK071637.1_Antioquia_10641/2015	95,54	95,42	96,23	99,66
MK071639.1_Cauca_2015	95,74	95,56	96,40	99,86
min-max	93,64 - 95,76	95,42 – 99,18	96,23 – 99,90	96,30 – 99,90

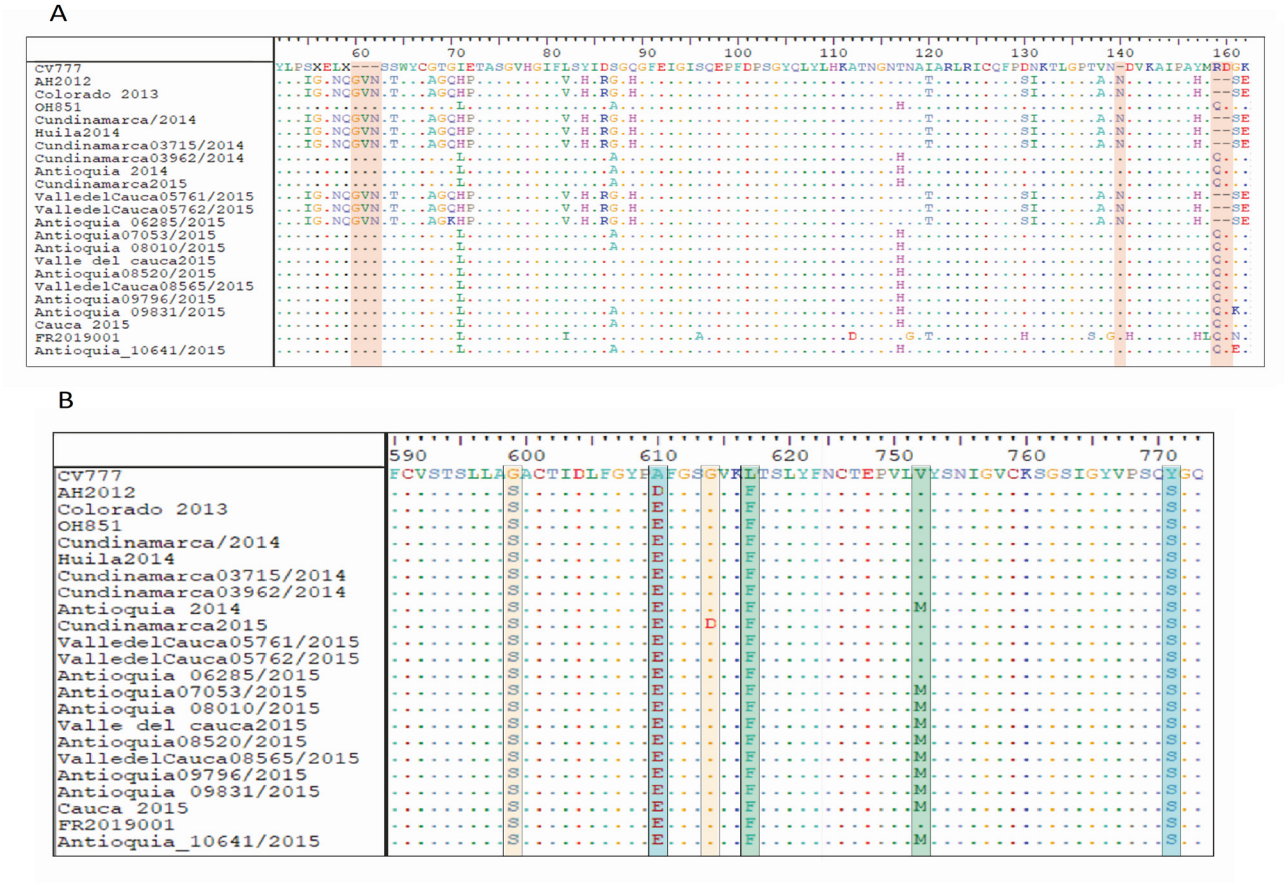

Figure 2. A: Insertions and deletions in the S1 domain (residue 1-725) of the spike gene for Colombian strains and reference strains. The insertions and deletions were highlighted in pink. B: Amino acid substitutions in neutralizing epitopes for the spike proteins of PEDV for the 18 Colombian strains compared to CV777 reference strain (A: Inserciones y deleciones en el dominio S1 (residuo 1-725) del gen Spike para cepas colombianas y cepas de referencia. Las inserciones y eliminaciones se resaltaron en rosa. B: Sustituciones de aminoácidos en epítopos neutralizantes para las proteínas de pico de PEDV para las 18 cepas colombianas en comparación con la cepa de referencia CV777).

In Europe, since 2014, PEDV re-emerged, including countries such as Germany (Hanke et al. 2015), the phylogenetic analysis of that study showed a very high nucleotide homology (99.5%) with the OH851 variant isolated in the US in 2014, that variant caused mild signs and low mortality rates in suckling piglets compared to the G2b strains. Additional reports of this S-INDEL strain have been published in other European countries such as Austria (Steinrigl et al. 2015), Belgium (Theuns et al. 2015), Portugal (Mesquita et al. 2015) and France (Grasland et al. 2015).

High homologies between non-S-INDEL strains from the US, other PEDV G1 and Taiwan strains were showed, including insertions of 4-aa (58QGVN62), 1-aa insertion (140N), and deletion of two amino acids (161 - 162) in the S protein, as compared to the CV777 vaccine strain and the historical HC070225-S strain from Taiwan. These similarities suggest a related pathogenesis between PED outbreaks in Taiwan and other countries during 2011-2014 (Chiou et al. 2017). In addition, Chinese strains identified in diarrheic pigs during 2011-2017 have insertions 59QGVN62, 140N, 157H, and 163NI164 deletions in the Spike gene, similar mutations have been observed in strains from South Korea that circulated in 2008-2009 (Chen et al. 2019).

Several epitopes for neutralizing antibodies have been identified for the S gene, among them, the epitope equivalent to CO-26K (COE epitope) (aa 575-639) (Chang et al. 2019), the epitopes SS2 (a. a. 748-755), SS6 (a. a. 764-771) and the porcine aminopeptidase N receptor binding domain (a. a. 490 ~ 615) (Wang et al. 2016); recently 3 epitopes (a. a. 527-559, 604-645, 677-734) in S1 domain (Zhu et al. 2019), and the neutralizing epitope within the S1A domain of protein S at 435-485 (Chang et al. 2019). In the multiple alignments performed at the present analysis for the CV777 reference strain and the Colombian strains, amino acid substitutions were observed for the following epitopes, COE (575-639): G599-S, A610-E, G614-D (Cundinamarca_2015), L617-F; SS6 (764-771): Y771-S and SS2 (748 – 755): V752-M. No amino acid deletions or insertions were observed in the epitopes for neutralizing antibodies.

CONCLUSION

In conclusion, these studies are useful to study the genetic background and the relationship between strains circulating in Colombia and global strains, which can be very important for the control and prevention of the disease. In the analysis, the strains showed a very

high nucleotide homology with strains reported in the US, suggesting the possible introduction of PEDV from the North American country. Studies and additional information are necessary to determine the origin of the strains reported in Colombia.

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