Virus Genes (2010) 40:225–230
DOI 10.1007/s11262-009-0433-3

Prevalence and phylogenetic analysis of the isolated type I porcine reproductive and respiratory syndrome virus from 2007 to 2008 in Korea

Chulseung Lee · Hyekwon Kim · Bokyu Kang · Minjoo Yeom · Sangyoon Han · Hyoungjoon Moon · Seongjun Park · Hyunil Kim · Daesub Song · Bongkyun Park

Received: 4 March 2009 / Accepted: 8 December 2009 / Published online: 9 January 2010

Abstract The first Korean strain of porcine reproductive and respiratory syndrome virus (PRRSV) was isolated in 1997, and it exhibited high similarity to strain VR-2332 (type II PRRSV; North American type). Recently, however, infection with type I PRRSV (European type) has also been reported in Korea. To date, preliminary data about type I PRRSV prevalence in Korea have not been reported. Here, using reverse transcriptase (RT)-PCR, we analyzed 383 archived field samples from 101 pig farms in Korea that were collected from 2007 to 2008. We identified 155 samples from 68 farms that were positive for PRRSV. Fifty-one samples (51/155; 32.9%) and 20 farms (20/68; 29.4%) were type I PRRSV-positive/type II PRRSV-negative. Furthermore, we tried to isolate the type I PRRSV from positive samples and seven type I PRRSV were isolated using PAM. The phylogenetic analysis using the type I PRRSV isolates (7 isolates) was performed based on open reading frame (ORF)5 (accession numbers GU325642 to GU325648) and ORF7 (accession numbers GU325635 to GU325641). In the phylogenetic study, seven type I PRRSV isolates were closely related with panEuropean based on ORF7, while they were genetically distinct from Lelystad virus and made a unique clade based on ORF5. The results of this study demonstrate that infection with type I PRRSV is not uncommon in Korean pig farms, which suggests that diagnosis and control of type I PRRSV should be considered in Korea. A new approach to vaccination against, and epidemiological analysis of, Korean PRRSV is urgently needed.

Keywords European PRRSV · Type I PRRSV · Phylogenetic analysis

Hyekwon Kim and Bokyu Kang contributed equally to this work.

Chulseung Lee, Hyekwon Kim, and Bokyu Kang are co-first authors.

C. Lee
Chung-Aang University School of Medicine, Seoul 156-756, Republic of Korea

H. Kim · B. Park (✉)
Department of Veterinary Medicine, Virology Laboratory, College of Veterinary Medicine and BK21 Program for Veterinary Science, Seoul National University, Gwanak-gu, Seoul 151-742, Republic of Korea
e-mail: parkx026@snu.ac.kr

B. Kang · M. Yeom · S. Han · H. Moon · S. Park · D. Song (✉)
Research Unit, Green Cross Veterinary Products, 227-5, Kugal-dong, Kiheung-gu, Yongin 449-903, Republic of Korea
e-mail: hfsong@daum.net; sds@snu.ac.kr

H. Kim
Optifarm Solution, Inc., 48 Songnam-Ri Seonggo-UB, Chunan City, Chungnam Province, Republic of Korea

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a small, enveloped, positive single-stranded RNA virus of the Arteriviridae family [4]. The causative agent of PRRS, PRRSV, was first described in Europe, where it was termed Lelystad virus [31], and in the United States, where it was termed VR-2332 [3, 6].

Porcine reproductive and respiratory syndrome virus emerged almost simultaneously in Europe and North America (type I and II PRRSV, respectively) and is associated with similar disease symptoms in both regions. However, type I and II PRRSV share only 55–70% identity in nucleotide gene sequence [7, 14, 16, 18, 19, 21–23]. Also, differences in virulence [9], and the antigenic [2, 23]

and genetic [19] properties of type I and type II viruses have been reported. This heterogeneity is likely to be one of the principal obstacles to effective prevention and control of the disease using commercial vaccines [17].

Lelystad and VR-2332 are considered to be the reference strains of type I and type II PRRSV, respectively. Additional PRRSV strains have been isolated from pigs at different times and in different geographical areas, and most have been partially sequenced. Phylogenetic analysis of PRRSV isolates has mainly focused on ORF5 [1, 8, 10, 15, 20, 29], which appears to be the most variable protein when type I and type II isolates are compared [12, 22]. In addition, ORF5 exhibits the highest degree of genetic diversity among viruses of the same genotype [24, 29].

The first Korean strain of PRRSV was isolated from the serum of an infected pig in 1997 [11]. The close phylogenetic relation of the isolate PL97-1 with type II strain VR-2332 indicated that the PL97-1 might be derived from the VR-2332-like strain. PL97-1 is genetically distant from the type I prototype, Lelystad virus. Thus, type II PRRSV strains that are widely separated by time and geography were placed together in early groupings of PRRSV [11].

Porcine reproductive and respiratory syndrome viruses that have been isolated in Asian countries, including Korea, are genetically distinct from more contemporary PRRSVs from the United States. Thus, PRRSVs identified in Asia have likely evolved independently of parental strains, resulting in the emergence of genetically distinct clades. Analysis of ORF5 reveals that sequence identity between Korean PRRSV isolates and VR-2332, the prototype type II PRRSV and the MLV vaccine virus, ranges from 87.1% to 98.7%, which corresponds to 85.1% to 98.0% identity at the amino acid level. This low level of sequence identity has raised concerns about the efficacy of current MLV vaccines [5].

There has been insufficient data relating with the prevalence and phylogenetic analysis of type I PRRSV in Korea. Here, we investigated the prevalence of type I PRRSV from 2007 to 2008 in Korea, and report the results of a phylogenetic analysis of Korean isolates based on the sequences of ORF5 and 7.

Materials and methods

Samples

A total of 383 samples from 68 farms, including lung tissue, were submitted to the Research Unit of Green Cross Veterinary Products from nationwide swine farms in 6 provinces of South Korea from 2007 to 2008. We collected the samples from 4- to 8-week-old illness pigs. The pigs usually showed clinical signs such as anorexia, lethargy, and abdominal breathing. And there was severe respiratory symptom in pigs.

Virus isolation

For virus isolation, lung specimens were collected and used to inoculate porcine alveolar macrophages (PAMs) isolated from the lungs of 50-day-old SPF piglets. Tissue homogenates were resuspended in Dulbecco's minimum essential medium (DMEM) (10% v/v) and the subjected to centrifugation. The supernatant was filtered (0.45-μm filter) and then used to inoculate PAMs. Viral isolates were passaged and analyzed by RT-PCR.

RT-PCR and sequencing

Viral RNA was extracted from tissue samples using Trizol LS, according to the manufacturer's instructions. RT-PCR was carried out using standard reaction conditions and a set of random hexamer primers.

Amplification of viral ORF5 and ORF7 sequences was carried out as described earlier [25]. Briefly, first, RT-PCR based on ORF7 was used to detect both types of viruses; then, RT-PCR based on ORF5 was used to differentiate type I and II PRRSV. Furthermore, amplified products of type I PRRSV isolates were purified using a QIAquick Gel Extraction kit (QIAGEN Korea Ltd.). Both strands of the purified product were sequenced by Genotech Co., Ltd. (Republic of Korea). All the sequencing reactions were performed in duplicate, and all the sequences were confirmed by sequencing both strands. The sequences of the isolated viruses were edited and analyzed using Bioedit software.

Phylogenetic analysis

The phylogenetic trees were generated using the MEGA 3.1 program and the ClustalX 3.81 alignment algorithm, with slight modifications. The Neighbor-joining tree was drawn using Kimura-two parameter as a distance estimation and percent frequencies of the groupings were determined after 1,000 bootstrap evaluation. Pairwise sequence alignments were also performed using the Bioedit program to determine nucleotide sequence similarities.

Results

Virus isolation and prevalence of PRRSV genotypes

We detected PRRSV in 155 samples (155/383, 40.5%) and 68 cases (68/101, 67.3%) by RT-PCR based on ORF7.

Table 1 Geographical prevalence of type I PRRSV among pig farms

Area	Genotype		
	EU	NA	EU + NA
Kyonggi	2[a]/5[b]	1/5	2/5
Chungcheong	3/5	2/5	–
Jeolla	7/25	11/25	5/25
Gangwon	1/1	–	–
Gyeongsang	1/9	7/9	1/9
Jeju	1/11	10/11	–
Unknown	5/12	6/12	–
Total	29.4% (20/68)	54.4% (37/68)	16.2% (11/68)

[a] Positive cases

[b] Total cases

Among PRRSV-positive samples or cases, Fifty-one samples (51/155, 32.9%) and 20 cases (20/68, 29.4%) were positive for type I PRRSV alone. Type II PRRSV alone was detected in 104 samples (104/155, 67.1%) and 37 cases (37/68, 54.4%). None of the samples were positive for both type I and type II PRRSV; however, there were some combined cases (11/68, 16.2%). At the level of Province, type I PRRSVs were detected all over the country (Table 1).

We isolated viruses of the type I genotype from seven of the 51 type I PRRSV-positive samples; each one isolate from Kyonggi, Chungcheong, and Gyeongsang, and two isolates from Jeolla and Gangwon provinces, respectively. We designated the seven viral isolates as follows: G2446-Korea-2008, G2448-Korea-2008, G210-Korea-2008, G221-Korea-2008, G301-Korea-2008, G302-Korea-2008, and G303-Korea-2008.

Sequence analysis of type I PRRSVs from Korea

We sequenced ORF5 and ORF7 from all the seven viral isolates, and then compared the sequences to available sequences in GenBank. Korean type I PRRSV ORF5 was 87.2–89.9% similar to Lelystad virus, and exhibited 93.2–99.1% similarity among the Korean type I PRRSV isolates. ORF7 was much more highly conserved than ORF5 in Korean type I PRRSVs, with 93.2–94.8% similarity to Lelystad virus, and 96.6–99.7% similarity among the Korean type I PRRSV isolates.

Phylogenetic analysis of ORF5

A neighbor-joining phylogenetic tree based on the sequence of ORF5 is shown in Fig. 1. Korean type I PRRSVs were related with type I PRRSVs from Western Europe, North America, and Thailand. Notably, Korean type I PRRSVs were grouped as a unique cluster, along with a PRRSV of Spanish origin (27-2003) [26]. The sequence identity showed that Korean type I isolates were 90.2–91.4% of identity with the Spanish type I PRRSV (27-2003).

Phylogenetic analysis of ORF7

A neighbor-joining phylogenetic tree based on the sequence of ORF7 is shown in Fig. 2. Korean type I PRRSV formed a unique subgroup when compared with PRRSV strains isolated from western countries, Thailand-and, and North America. Korean isolates were more closely related to panEuropean subtype than esternEuropean subtype. 80.0–86.2% of sequence identity was observed between Korean type I PRRSV and esternEuropean subtype viruses from Belarus and Russia, while 88.8–94.8% of sequence identity was observed between Korean type I PRRSV and panEuropean PRRSVs based on ORF7.

Deduced amino acids of ORF5 in Korean type I PRRSV

The amino acids of Korean type I PRRSV had specific changes compared to the Lelystad virus (Fig. 3). The conserved changes were found at different regions (37D → 37N, 56D → 56A/T, 40S → 40T, 48G → 48S/P, 100T → 100I, 101A → 101T, 111C → 111S, 113V → 113I, 119F → 119L, 126V → 126A, 154V → 154I, 161I → 161V, 171V → 171I). However, the putative cleavage sites and neutralizing epitope were found to be highly conserved [32].

Discussion

On the basis of RT-PCR analysis, the incidence of type I PRRSV infection is not uncommon in Korea. The positive rate of type I PRRSV was 32.9% among individual pigs and 29.4% among swine farms from 2007 to 2008. When farms with both type I and II infections are factored in, 46.6% of swine farms are positive for type I PRRSV. This result was outstanding because the first sequence data about type I PRRSV was just reported at GenBank in 2005, which meant that the spread of type I PRRSV was rapid in Korea. Previously, it was reported that type II PRRSV vaccines do not effectively reduce viremia after challenge with wild-type type I PRRSV [30]. In Korea, the currently available commercial PRRSV vaccine targets only type II PRRSV, and the current MLV vaccine does not evoke protective antibodies against pathogenic type I PRRSV. These results highlight the importance of strategies to develop novel type I PRRSV vaccines.

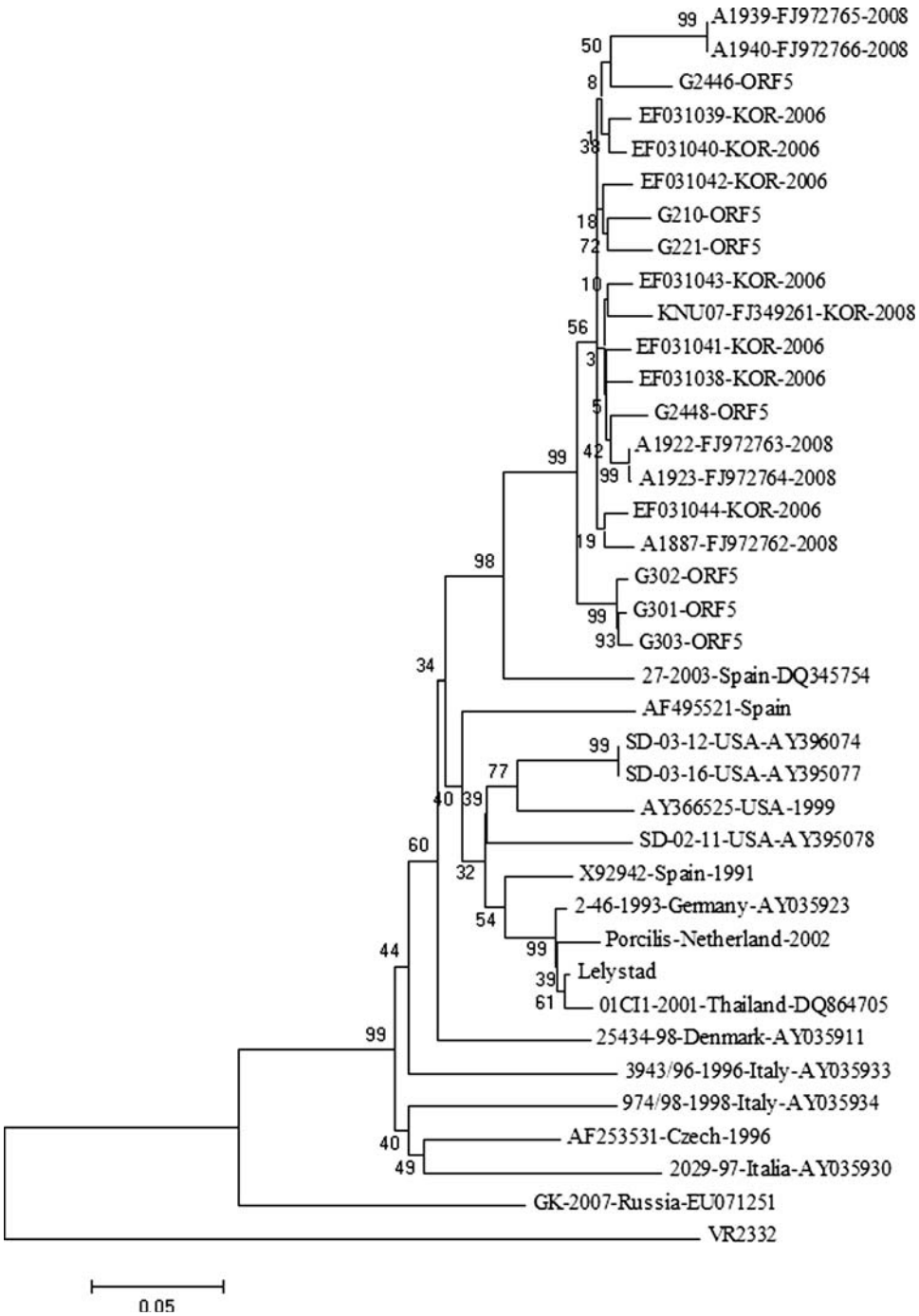

Fig. 1 Neighbor-joining phylogenetic tree based on the sequences of ORF5 of type I PRRSV isolates in Korea. All the reference sequences were indicated by GenBank accession numbers

Phylogenetic analysis of seven type I PRRSV isolates revealed that these type I PRRSVs form a distinct clade from previously reported viruses in Europe, North America, and Thailand (Figs. 1, 2). On the basis of ORF5, the seven isolated type I PRRSVs were also in the same group with previously reported Korean type I PRRSV from 2006 to 2008, showing 96.6–99.7% of identity. Although these viruses were in the same subgroup with Spanish isolate (27-2003), sequence identity was only 90.2–91.4%. This genetic closeness among Korean type I PRRSV led us develop a "Korean type I PRRSV"-specific vaccine with the isolated viruses. However, when using the isolated viruses, large production system such as cell-line cell adaptation should be developed.

On the basis of ORF7, type I PRRSVs can be subdivided into at least three subtypes (panEuropean subtype 1, esternEuropean subtypes 2 and 3) in European countries [27, 28]. In this study, Korean type I PRRSVs was in panEuropean subtype. Although Korean type I PRRSVs were shown to belong to panEuropean subtype in the phylogenetic tree

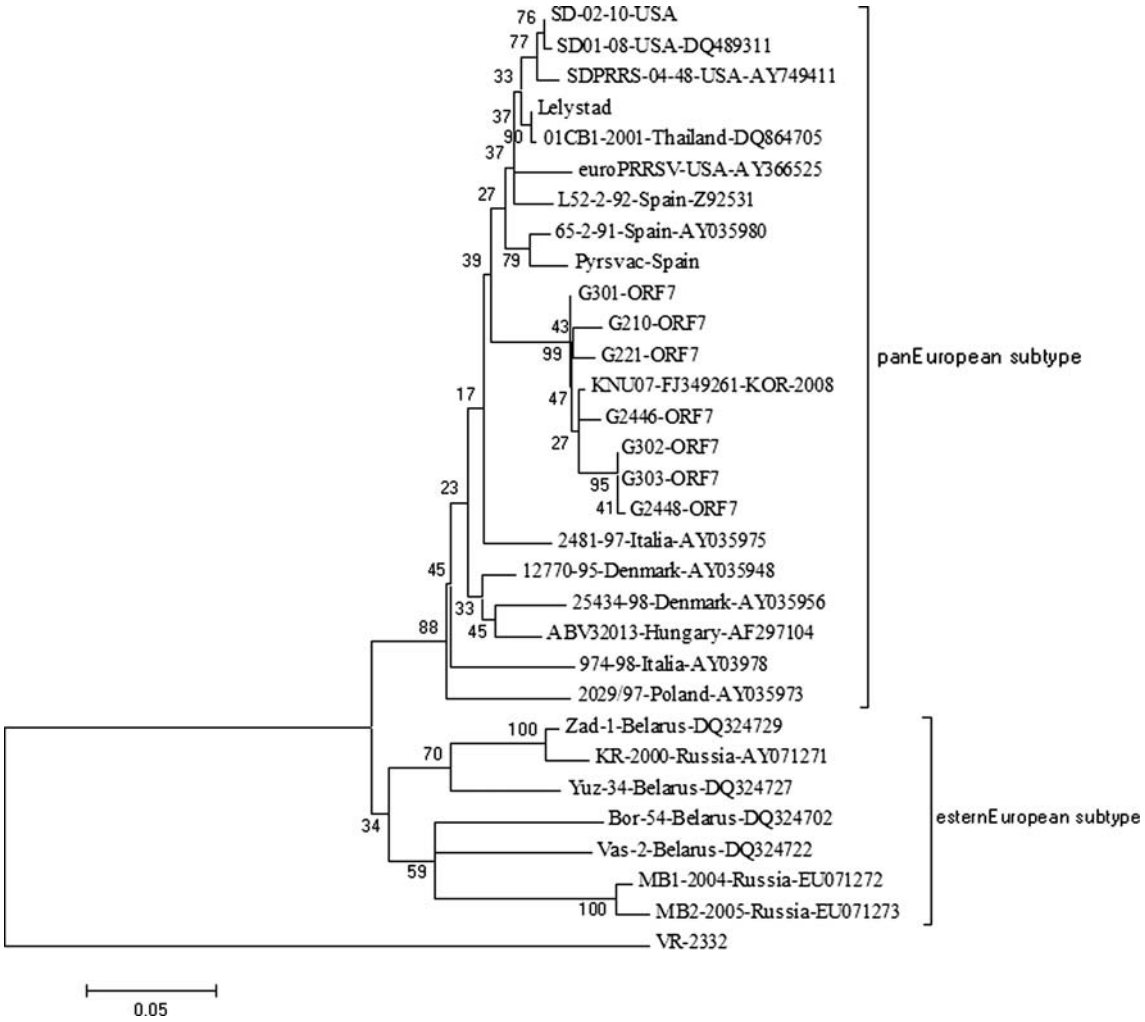

Fig. 2 Neighbor-joining phylogenetic tree based on the sequences of ORF7 of type I PRRSV isolates in Korea. All the reference sequences were indicated by GenBank accession numbers. Previously reported reference sequences of panEuropean subtype and esternEuropean subtype were included in this study [28]

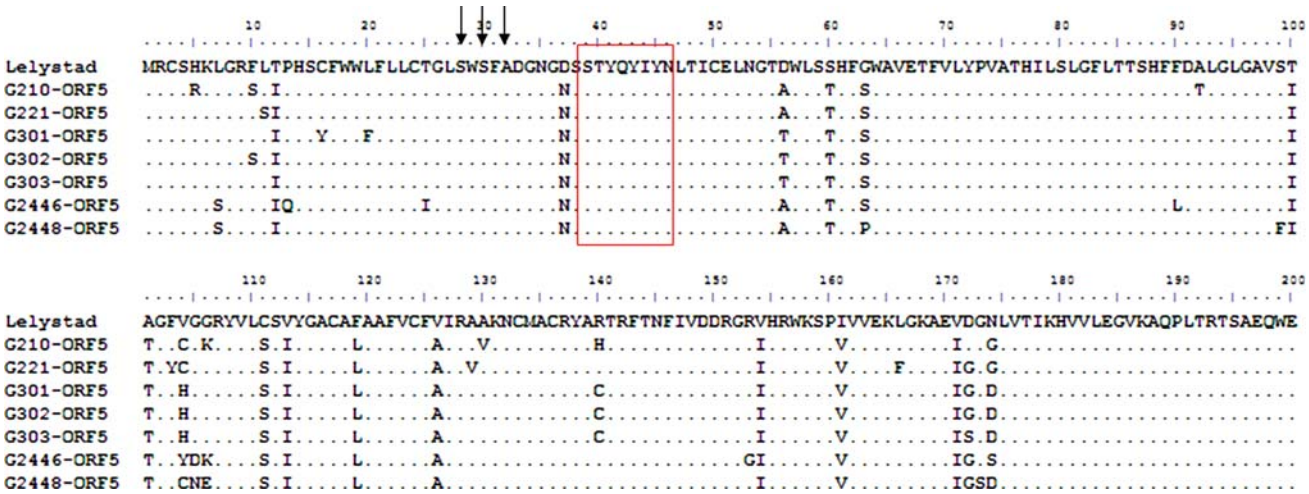

Fig. 3 Comparison between deduced amino acids of ORF5 in Korean type I PRRSV and Lelystad virus. A total of 200 amino acid sequences were presented for the comparison. A *box* and *arrows* indicated the neutralizing epitope and putative cleavage sites, respectively

based on ORF7, they were genetically distinct from Lelystad virus based on ORF5, and type I PRRSVs from USA, which indicates that type I PRRSV evolved independently in Korea, resulting in the emergence of a genetically distinct clade. As a previous study about type I PRRSV based on ORF5, [13] this study using type I PRRSV isolates based on ORF5 and ORF7 suggests that Korean type I PRRSVs were introduced in Korea far earlier than 2006, and evolved independently on a nationwide scale.

Our results raise concerns that type I PRRSV infections are not uncommon in pigs in Korea as well as type II PRRSV infections, and highlight the need for monitoring type I PRRSV in pig farms. Even though there also has been a concern about type II PRRSV-specific vaccines, a PRRSV vaccine that targets type I PRRSV is urgently needed.

Acknowledgments This study was supported by Technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea.

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