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SEROTYPES OF DENGUE VIRUSES CIRCULATING IN JAZAN REGION, SAUDI ARABIA

By

ADEL A. ALSHEIKH[1*], O. M. DAFFALLA[1], E. M. NOURELDIN[1], W. S. MOHAMMED[1], K. J. SHRWANI[1], Y. A. HOBANI[1], A. D. ABKAR[2], F. A. ALSHEIKH[3] AND A. M. ASSIRI[4]

National Center for Vector-Borne Diseases[1], MoH, Jazan, Department of Medical Parasitology[2], Faculty of Medical Laboratory Sciences, University of Gezira, Sudan[3], Faculty of Public Health, Jazan University, and Minister Deputy Assistant for Preventive Health[4], MoH-Riyadh, Saudi Arabia[1,2,4]

(Correspondence: adelalsheikh@gmail.com)

Abstract

Dengue fever is considered to be the most important mosquito-borne disease and considered as endemic disease in Jazan region, Saudi Arabia**.** The present study aimed to analyze the prevailing dengue virus serotypes in the region. Serum samples of 220 suspected dengue cases were collected throughout 2016 and tested by one step Reverse Transcription Polymerase Chain Reaction (RT-PCR) with a set of specific primers for detection of four DV serotypes followed by sequencing the PCR products to confirm the results. Out of the 220 serum samples, 124 were found positive for dengue infection (56.4%). Three dengue virus serotypes were detected; DEN-1, DEN-2 and DEN-3. DEN-2 is the most common and predominant type in the region rating 83.9% (104/124), followed by DEN-1 8.9% (11/124), and then DEN-3 7.2% (9/124). The high seroprevalence of dengue virus infections in Jazan region indicates its endemicity. The present study highlights the importance of tracking the spread of dengue virus types and its implication for analyzing changes in dengue endemicity in specified areas over time.

Key words: Dengue fever, Serotypes, 1, 2 & 3, Jazan region, Saudi Arabia.

Introduction

Dengue fever is considered to be the most important re-emerging vector-borne disease worldwide and is endemic in more than 125 countries (Murray *et al*, 2013). Four hundred million of cases are estimated to occur annually (CDC, 2016). Dengue is a viral disease transmitted to humans by the bite of infected females of the main vector *Aedes aegypti* and to a lesser extent by *Aedes albopictus* mosquitoes (Alto *et al*, 2008). There were five genetically related but antigenically distinct single-stranded RNA serotypes belongs to *flaviviridae* family and genus *flavivirus*; DEN-1, DEN-2, DEN-3, DEN-4 (WHO, 1997), and DEN-5 (Mustafa *et al*, 2015). However, no cross protection occurred between dengue serotypes, the immunity is serotype specific. According to disease severity, WHO (1997) classified dengue into three categories; Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF), and Dengue Shock Syndrome (DSS). Unplanned urbanization and climatic factors, including high temperatures and rainfall, might contribute to epidemics of dengue (Mackenzie *et al*, 2004; Crowell *et al*, 2011; Banu *et al*, 2011).

Aedes mosquito is found in urban settings, mainly in tropical areas (Heikal *et al*, 2011; Shoukry et al, 2012), maintaining a sustainable relationship with man led to reemergence of dengue infections and creating a public health threat (Glenn and Sia, 2008).

Spatial patterns in the recent and sequential circulation of DEN1-5, along with the host and virus genetics, should be regarded as potential population risk factors for sever forms of dengue fever (Guilarde *et al*, 2008; Chaturvedi *et al*, 2006), because most secondary infection bearing heterologous dengue virus type may lead to severe disease complications (Vaughn *et al*, 2000; Gibbons and Vaughn, 2002; Rico-Hesse, 2003; Green and Rothman, 2006; Guilarde *et al*, 2008; El-Bahnasawy *et al*, 2011).

In Saudi Arabia, the first dengue outbreak has been reported in 1994 in Jeddah with 289 confirmed cases, and DEN-1 and DEN-

2 were circulating dengue virus serotypes (Fakeeh and Zaki, 2001). Since then, several dengue fever outbreaks have been recorded in Saudi Arabia (Fakeeh and Zaki, 2003; Ahmed, 2010; Khan *et al*, 2008; Ayyub *et al*, 2006; El-Badry *et al*, 2013; Zaki *et al*, 2008) and Yemen (Madani *et al*, 2013). The case fatality rate was 4.6 per thousand in 2007 (Saudi Ministry of health, 2007).

The incidence of dengue fever has increased in Saudi Arabia during the past few years; 6512 cases in the year 2013; 2081 cases in year 2014; and 4312 cases in year 2015 (Saudi Ministry of health, 2016).

The emergence of DENV-3 in Jeddah was in 1997 (Fakeeh and Zaki, 2001), and since then all the 3 dengue serotypes (DENV 1-3) were being circulated in the city (Azhar *et al*, 2015).

Organji *et al* (2017) reported that DEN-1, DEN-2, and DEN-3 to be circulated in Makkah City. In Jazan Region, there were 1790 confirmed dengue cases between years 2005 and 2016 with highest outbreaks in 2016 (555 cases), followed by 2010 (290 cases), and 2012 (289 cases) (Dengue control program in Jazan). Al-Arzaqi *et al.* (2013) reported dengue prevalence of up to 26.5% in Jazan Region, while Gamil *et al.* (2014) noted 47.74% dengue positivity rate in the area.

To the best of the authors' knowledge, no data was published on the circulation of the dengue virus serotypes in Jazan Region, thus thr present study is the first of its own and aimed to analyze the prevalence of dengue virus serotypes circulating in Jazan Region, southwest of Saudi Arabia.

Materials and Methods

Study area: Jazan Region lies in Southwest Saudi Arabia between 16^{o}-12, and 18^{o}-25, latitude north. It is bordered in the South by Arabic republic of Yemen with total area of about 22,000km² and 1.3 million populations (census 2011). Thirty percent of the population is concentrated in six major cities, and the remainders living in over 3500 villages (Alsheikh, 2011). Jazan Region is situated in the subtropical zone and has the average monthly temperatures ranged between 25.8°C in January to 33.4°C in July, and average relative humidity ranged between 55% & 72.5%. The rainy season is started at August through October with a monthly average of 77 & 56.7mm, respectively (Alsheikh, 2011). Jazan is divided into eleven small Governorates (Al-Aridah, Damad, Twal, Al-Ahad, Jazan, Al-Khobah, Samttah, Abuareesh, Sabyah, Beash and Al-Darb), these locations (Fig.1) although with different altitudes and geographical characteristics, they are almost sharing the same demographical, agricultural, educational, cultural, housing, health system, and environmental characteristics.

Sampling: During the year 2016, about 220 suspected dengue fever patients sera included in the study were collected from five different hospitals in Jazan and stored at -80^{o} C till further used.

RNA isolation: High Pure Viral Nucleic Acid Kit from Roche applied science (Germany) used for extraction of RNA follow the manufacture procedure; 200µl of bind ing buffer supplemented with poly (A) and 50µl Proteinase K added to 200µl of serum sample then mixed immediately and incubated for 10 minutes at 72^{o}C. The addition of 100µl Proteinase K was mixed with sample and transferred to High Filter Tube inserted into Collection Tube. After centrifugation for 1 minute at the 10000rpm, the collection tube was discarded. The filter tube combined with the new collection tube and 500µl of inhibitor removal buffer was added and centrifuged for 1 minute at 10000 rpm. After changing collection tube, the high filter tube washed twice by adding 450µl of wash buffer at the same condition of centrifugation, followed by centrifugation for 15 seconds at 13000 rpm to remove any residual wash buffer. Then the high filter tube was inserted into nuclease free, sterile 1.5ml centrifuge tube and 50µl of elution buffer was added to elute the viral nucleic acid by centrifugation at 10000 rpm for 1 minute.

Reverse Transcriptase Polymerase Chain

Reaction (RT-PCR): One step RT-PCR proved a rapid, sensitive, and simple for dengue serotype-specific diagnosis method. The test was performed after the protocol of Lanciotti *et al.* (1992) with some modification; The DEN consensus primers and serotype-specific primers (Tab. 1) were used to amplify the viral genome and synthesized in Integrated DNA Technology (Belgium). The one step RT-PCR reactions was performed according to access RT-PCR-system protocol (Promega, USA) in a volume of 50µl containing 10µl of AMV/Tfl 5X Reaction Buffer, 1µl of dNTP Mix (10mM each dNTP, final concentration 0.2mM), 2µl of 25mM $MgSO_4$ (final concentration 1mM), 1µl of AMV Reverse Transcriptase 5u/µl (final concentration 0.1u/µl), 1µl of Tfl DNA Polymerase 5u/µl (final concentration 0.1u/µl), 50pmol (final concentration 1µM) of each forward (D1) and reverse (D2) primers, 5µl of RNA virus and nuclease free water to total volume 50µl. The thermal cycling incubations temperatures programmed as follows: incubation for an hour at 42^{o}C (to convert the RNA to cDNA) then initial denaturation for 3 minutes at 94^{o}C followed by 35 cycle of denaturation (94^{o}C, for 30 second), primers annealing (55^{o}C for 1 minute), primer extension (72^{o}C for 2 minutes) and final extension for 5 minutes.

Nested-PCR: Nested PCR was performed in 2 tubes for each sample in 50µl reaction mixture containing 25µl GoTag®G2 green master mix ready to use from Promega, 10µl of diluted (1:100) RT-PCR product, 50pmol (final concentration 1µM) of each forward primer D1 and TS1, TS3 as reverse primers for the first tube and TS2, TS4 as reveres primers for another tube. The samples were subjected to initial denaturation at 94^{o}C for 3 minutes, 30 cycles of denaturation (94^{o}C, 30 s), primer annealing (55^{o}C, 1 min), primer extension (72^{o}C, 2 min) and final extension for 5 minutes. In each run negative and positive controls were included. PCR products of nested amplification were analyzed by gel electrophoresis (1.5 agarose in Tris-Acetate EDTA buffer) staining with ethidium promide. The visualization was carried out using Gel Doc XR Imaging System (Bio-Rad).

Fig. 1: Map of Jazan Region showing different Governorates.

Table 1: oligonucleotide primers used in RT-PCR and Nested-PCR

primer	Sequence 5 - 3	Genome position	Size in bp
D1	TCAATATGCTGAAACGCGCGAGAAACCG	134-161	511
D2	TTGCACCAACAGTCAATGTCTTCAGGTTC	616-644	511
TS1	CGTCTCAGTGATCCGGGGG	568-586	482 (Dl and TS1)
TS2	CGCCACAAGGGCCATGAACAG	232-252	119 (Dl and TS2)
TS3	TAACATCATCATGAGACAGAGC	400-421	290 (Dl and TS3)
TS4	CTCTGTTGTCTTAAACAAGAGA	506-527	392 (Dl and TS4)

Sequencing and bioinformatics analysis: Purification and standard sequencing for RT-PCR products were performed by Macrogen Co. (Seoul, Korea). Sequencing reactions were done in a MJ Research PTC-225 Peltier Thermal Cycler using a ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems), following the protocols supplied by the manufacturer. Single-pass sequencing was performed on each template using D1 (forward) primer. The fluorescent-labeled fragments were purified from the unincorporated terminators with Big Dye®X Terminator™ purification protocol. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems). Sequences were searched for sequence similarity through BLAST (www.ncbi.nlm.nih.gov/BLAST) (Atschul *et al*, 1997) and compared to reference sequences of Dengue serotypes detected in BLAST and downloaded from GenBank (www.ncbi.nlm.nih.gov/genbank). Similarity tree was obtained from database online by phylogeny.fr (http://www. phylogeny.fr/).

Results

RT-PCR and Nested-PCR: One hundred twenty four samples of 220 (56.4%) suspected patient serum samples tested by RT-PCR were confirmed positive for dengue virus when using D1 & D2 primers (511bp) for all serotypes, and RT-PCR product was used as a sample for the nested-PCR using a set of serotype-specific primers pair as described in methodology. Three dengue virus types (DEN-1, DEN-2 & DEN-3) were detected and the results showed that DEN-2 was the most common and predominant type in Jazan Region rating one hundred four out of one hundred twenty four (83.9%), followed by DEN-1 (eleven out of one hundred twenty four, 8.9%), and then DEN-3 (nine of one hundred twenty four, 7.2%) and serotype 4 was not detected (Tab. 2; Fig. 2).

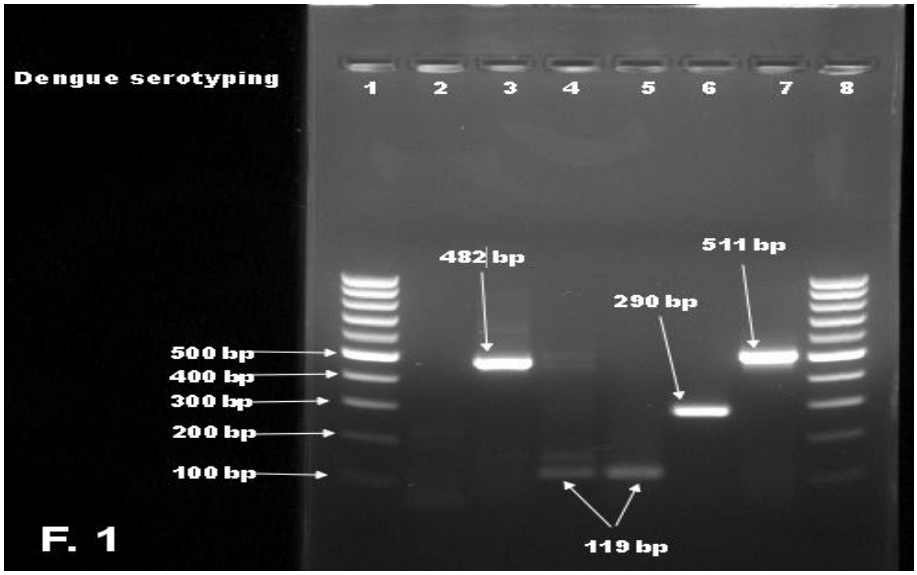

Fig. 2: Agarose gel electrophoresis of RT-PCR (D1, D2 primers) and nested-PCR by specific primers. Lane 1 and 8 DNA 100bp marker, lane (2) negative control, lane (3) positive sample DENG 1, lane (4,5) positive samples DENG2, lane (6) positive sample DENG3 and lane (7) positive RT-PCR product sample (D1 and D2 primers for all serotypes).

Table 2: Dengue virus serotyping using RT-PCR and nested-PCR

No of samples	$^{+}$ve DENG	$^{+}$ve DEN-1	$^{+}$ve DEN-2	$^{+}$ve DEN-3	$^{+}$ve DEN-4
220	124 (56.4%)	11 (8.9%)	104 (83.9%)	9 (7.2 %)	0 (0%)

Sequencing: Partial sequencing was done for 19 RT-PCR product samples of DEN-1, DEN-2, & DEN-3. Blast search showed sequences of samples aligned along with published sequences of dengue virus serotypes (Tab. 3; Figs. 3, 4 & 5) and similar tree (Figs. 6, 7 & 8) that illustrated Gen-bank accession numbers and country's isolates.

Table 3: RT-PCR and nested-PCR

DEN 1		DEN 2		DEN 3	
Gen bank accession No.	Country	Gen bank accession No	Country	Gen bank accession No	Country
AB608788	Taiwan	JN935383	India	KM097092	India
KJ649286	Saudi Arabia	KU351296	India	KM097092	Singapore
JN638338	Thailand	GU968539	India	KF954949	China
AF298808	Djibouti	KX577706	China	GQ466079	India
Z74047	Vietnam	KT180256	India	FJ644564	India
AF538024	Cambodia	KU351306	India	DQ317393	India
KU509258	Eritrea	JQ639472	India	KU216208	India

Query 11 ACTGTTTC-CAGTTGGCG-AGAGATTCTCAAAAGGATTGCTCTCAGGCCAAGGACCCATG 68
Sbjct 111 ACTGTTTCACAGTTGGCGAAGAGATTCTCAAAAGGATTGCTCTCAGGCCAAGGACCCATG 170
Query 69 AAATTGGTGATGGCTTTCATAGCATTCCTAAGATTTCTAGCCATACCCCCAACAGCAGGA 128
Sbjct 171 AAATTGGTGATGGCTTTCATAGCATTCCTAAGATTTCTAGCCATACCCCCAACAGCAGGA 230
Query 129 ATTTTGGCTAGATGGGGCTCATTCAAGAAGAATGGGGCGATTAAAGTGTTACGGGGTTTC 188
Sbjct 231 ATTTTGGCTAGATGGGGCTCATTCAAGAAGAATGGAGCGATTAAAGTGTTACGGGGTTTC 290
Query 189 AAAAAAGAAATCTCAAACATGTTGAATATAATGAACAGAAGGAAGAGATCCGTGACCATG 248
Sbjct 291 AAGAAAGAAATCTCAAACATGTTGAATATAATGAATAGAAGGAAGAGATCCGTGACCATG 350
Query 249 CTCCTCATGCTGCTGCCCACAGTCTTGGCGTTCCATCTGACCACACGAGGGGGAGAGCCG 308
Sbjct 351 CTCCTTATGCTGCTGCCCACAGTCTTGGCGTTTCATCTGACCACACGAGGGGGAGAGCCG 410
Query 309 CATATGATAGTCACCAAGCAGGAAAGAGGAAAGTCACTTTTGTTTAAGACTTCAACTGGT 368
Sbjct 411 CATATGATAGTTACCAAGCAGGAAAGAGGAAAGTCACTTTTGTTTAAGACCTCAGCTGGT 470
Query 369 GTCAACATGTGCACCCTTATCGCAATGGATTTGGGAGAGTTATGTGAGGACACAATGACT 428
Sbjct 471 GTCAACATGTGCACCCTTATTGCGATGGATTTGGGAGAGTTATGTGAGGACACAATGACT 530
Query 429 TACAAATGTCCCCGAATCACTGAGGCGGAACCTGAAGACATTGAT-GTTTGGTGCAA 484
Sbjct 531 TACAAATGTCCTCGAATCACTGAGGCGGAACCAGATGACGTTGATTGTT-GGTGCAA 586
Query = Sample, Sbjct = Reference.

Fig. 3: Identities between DEN-1 from Jazan and DEN-1 of Taiwan (dbj|AB608788.1). Dengue virus 1 gene for polyprotein, complete cds, strain: 832, Length=10693, Score = 778 bits (421), Expect = 0.0, Identities = 459/477 (96%), Gaps = 4/477 (1%), Strand= Plus/Plus.

Query 8 ACTGTGC-ACAGCTGAC-AAGAGATTCTCACTTGGAATGCTGCAGGGACGAGGACCGTTG 65
Sbjct 34ACTGTGCAACAGCTGACAAAGAGATTCTCACTTGGAATGCTGCAGGGACGAGGACCGTTG 93
Query 66 AAACTGTTCATGGCCTTGGTGGCATTCCTTCGTTTCCTAACAATCCCGCCAACAGCGGGG 125
Sbjct 94 AAACTGTTCATGGCCTTGGTGGCATTCCTTCGTTTCCTAACAATCCCACCAACAGCAGGG 153
Query 126 ATACTAAAAAGATGGGGAACGATCAAAAAGTCAAAAGCCATCAATGTCTTGAGAGGGT TC185
Sbjct 154 ATACTAAAAAGATGGGGAACGATCAAAAAGTCAAAAGCCATCAATGTCTTGAGAGGGTT C 213
Query 186 AGGAAAGAGATTGGAAGGATGTTGAACATCTTGAATAGGAGACGCAGAACTGCAGGC GTG 245
Sbjct 214 AGGAAAGAGATTGGAAGGATGTTGAACATCTTGAATAGGAGACGCAGAACTGCAGGCG-TG 273
Query 246 ATCATCATGCTAATTCCAACAGCGATGGCGTTCCATTTAACCACACGCAACGGAGAACC A 305
Sbjct 274 ATCATCATGCTAATTCCAACAGCGATGGCGTTCCATTTAACCACACGCAACGGAGAACCA 333
Query 306 CACATGATCGTCAGCAGACAAGAGAAAGGGAAAAGTCTCTTGTTCAAAACAGAGGATG GT 365
Sbjct 334 CACATGATCGTCAGCAGACAAGAGAAAGGGAAAAGTCTCTTGTTCAAAACAGAGGATGG GT 393
Query 366 GTGAACATGTGTACCCTCATGGCCATGGACCTTGGTGAACTGTGTGAAGACACAATCA CT 425
Sbjct 394 GTGAACATGTGTACCCTCATGGCCATGGACCTTGGTGAACTGTGTGAAGACACAATCACT 453
Query 426 TATAACTGTCCTCTTCTCAGGCAGAATGAACCTGAAGACATTGACTGTTTGGTGCa 481
Sbjct 454 TATAACTGTCCTCTTCTCAGGCAGAATGAACCTGAAGACATTGACTGTT-GGTGCA 508
Query = Sample, Sbjct = Reference.

Fig. 4: Identities between DEN-2 from Jazan and DEN-2 of India (gb|JN935383.1). Dengue virus strain VCRC/ DENV2/03/10 polyprotein gene, partial cds Length=508, Score = 848 bits (459), Expect = 0.0, Identities = 471/476 (99%), Gaps = 3/476 (1%), Strand=Plus/Plus

Query 7 ACTGGATCACAGTTGGCG-AGAGATTCTCAAAAGGATTGCTGAACGGCCAGGGACCAATG 65
Sbjct 151 ACTGGATCACAGTTGGCGAAGAGATTCTCAAAAGGATTGCTGAACGGCCAGGGACCAATG 210
Query 66 AAATTGGTCATGGCGTTCATAGCCTTCCTTAGATTTCTGGCCATTCCACCAACAGCAGGA 125
Sbjct 211 AAATTGGTCATGGCGTTCATAGCCTTCCTTAGATTTCTGGCCATTCCACCAACAGCAGGA 270
Query 126 GTTTTGGCCAGATGGGGAACCTTCAAGAAGTCGGGTGCCATTAAGGTTCTGAAAGGCTTC 185
Sbjct 271 GTTTTGGCCAGATGGGGAACCTTCAAGAAGTCGGGGGCCATTAAGGTTCTGAAAGGCTTC 330
Query 186 AAGAAGGAGATTTCAAACATGCTGAGCATAATCAACAAACGGAAAAAGACATCGCTCTGT 245
Sbjct 331 AAGAAGGAGATTTCAAACATGCTGAGCATAATCAACAAACGGAAAAAGACATCGCTCTGT 390
Query 246 CTCATGATGATATTGCCAGCAGCACTTGCTTTCCACTTGACTTCACGAGATGGAGAGCCG 305
Sbjct 391 CTCATGATGATATTGCCAGCAGCACTTGCTTTCCACTTGACTTCACGAGATGGAGAGCCG 450
Query 306 CGCATGATTGTGGGGAAGAATGAAAGAGGAAAATCCCTACTTTTTAAGACAGCCTCTGGA 365
Sbjct 451 CGCATGATTGTGGGGAAGAATGAAAGAGGAAAATCCCTACTTTTTAAGACAGCCTCTGGA 510
Query 366 ATCAACATGTGCACACTCATAGCCATGGACTTGGGAGAAATGTGTGATGACACGGTCACT 425
Sbjct 511 ATCAACATGTGCACACTCATAGCCATGGACTTGGGAGAAATGTGTGATGACACGGTCACT 570
Query 426 TACAAATGCCCCCACATTACCGAAGTGGAACCTGAAGACATTGACTGTTGGTGCAA 481
Sbjct 571 TACAAATGCCCCCACATTACCGAAGTGGAACCTGAAGACATTGACTGCTGGTGCAA 626
Query = Sample, Sbjct = Reference.

Fig. 5: Identities between DEN-3 from Jazan and DEN-3 of India (gb|KF954949.1) Dengue virus 3 isolates 13GDZDVS30E, complete genome Length=10677 Score = 861 bits (466), Expect = 0.0, Identities = 473/476 (99%), Gaps = 1/476 (0%, Strand=Plus/Plus

Discussion

Jazan Region has witnessed several outbreaks during the recent decade (290 cases in 2010, 289 cases in 2012, and 555 cases in 2016- Dengue control program in Jazan).

The current available data on dengue in Jazan has concentrated mainly on serological surveys (Al-Arzaqi *et al*, 2013; Gamil *et al*, 2014) and has not analyzed the circulating serotypes in the region.

The present results showed that dengue fever is becoming highly prevalent in Jazan region (56.4%) compared to the previous reports of Al-Arzaqi *et al.* (2013) and Gamil *et al.* (2014) who reported dengue prevalence of 26.5% and 47.74%, respectively, in the region. In this study, three dengue virus types (DEN-1, DEN-2 & DEN-3) were found circulating in Jazan Region with the predominance of DEN-2 scoring 104 out of 124 dengue positive samples (83.9%), followed by DEN-1 (11/124-8.9%), and DEN-3 (9/124-7.2%), however serotype 4 was not detected in any of the 124 dengue cases. This finding is in complete agreed with Fakeeh and Zaki (2001) who reported that DEN-2 was the predominant serotype, followed by DEN-1, and DEN-3 in Jeddah, Saudi Arabia. Whereas Organji *et al.* (2017) in Makkah City, showed that the DEN-1 was the predominant dengue virus type, followed by DEN-2 and then DEN-3, although the positive blood samples they used were only six. The result was also coincided partially with the findings of Khan *et al.* (2008) who reported high prevalence of the DEN-2 in contrast to the DEN-2 in Makkah City.

In Jeddah, Zaki *et al.* (2008) revealed that the DEN-1 and DEN-2 caused the major outbreak in 1994, while DEN-3 emerged in year 1997. Besides, they indicated two genotypes for DEN-1 (America-Africa genotype, and Asia-2 genotype), DEN-2 genotype clu-0stered within Cosmopolitan genotype, and DEN-3 clustered within genotype III.

In the present study, it was found the DEN-2 was the predominant dengue virus type, a result which is in line with the reports of Fakeeh and Zaki (2001, 2003) and Zaki *et al.* (2008) who stated that DENV-2 virus is the predominant serotype in Saudi Arabia particularly in western Saudi Arabia since 1992. El-Kafrawy *et al.* (2016) showed that DEN-2 isolate from Jeddah belongs to the Cosmopolitan genotype was most genetically related to isolates from India (Chatur-

vedi *et al*, 2006) and Pakistan circulating from 2007 to 2013 (Hasan *et al*, 2014).
The dengue virus serotypes DEN-1, DEN-2, & DEN-3 were thought to be predominant in the Middle East, especially in Yemen and Saudi Arabia (Nedjadi *et al*, 2015).

Dengue viruses circulating locally in Saudi Arabia were likely to have been imported into Saudi Arabia by Saudi traveling abroad to dengue endemic countries, or during the Hajj and Umrrah seasons, or by the immigrant workers (Zaki *et al*, 2008).

The introduction of the three dengue virus types in Jazan Region might be resulted from several factors; such as traveling of the Jazani citizens for the Hajj and/or Umrrah (Al-Azraqi *et al*, 2013), or for trade or other purposes, or by traveling abroad to dengue endemic countries as Bangladesh, India, Indonesia, Philippines, Singapore, Sri Lanka, Thailand (Toan *et al*, 2015), and/or Malaysia (Packierisamy *et al*, 2015), or by migrant workers, or due to the proximity of Jazan to Yemen where the disease is endemic (Ciccozzi *et al*, 2014). Besides, a total of 22 countries in Africa reported sporadic cases or outbreaks of dengue; 12 other countries in Africa reported dengue only in travelers (Amarasinghe *et al*, 2011).

It was stated that shifts in circulating dengue virus type or introduction of new dengue virus type in endemic areas have shown to be related with the incidence of severe dengue infections; DHF & DSS (Messer *et al*, 2003; Rico-Hesse *et al*, 1997). Moreover, it was worthy to note that the primary infections by DEN-1 and DEN-3 were related with the more dengue severe infections, whereas infections with DEN-2 and DEN-4 were associated with increased dengue severity when they presented as the secondary infections (Balmaseda *et al*, 2006).

Generally speaking, the rapid geographical expansion of dengue (Murray *et al*, 2013) has been paralleled by an increase in dengue research and research funding (Wilder-Smith and Schwartz, 2005). This increase was further spurred by the perceived threat of dengue to currently non-infected areas, including the threat to Western countries. Imported dengue cases via the international travellers to the Western countries were rising (Wilder-Smith and Gubler, 2008).

The present results were able to analyze multiple dengue serotypes which would help in providing clear evidence of current active dengue transmission and its endemicity in Jazan Region.

Conclusion

The results of this study reported for the first time the dengue virus types DEN-1, DEN-2, and DEN-3 circulating in Jazan region with the DEN-2 being the predominant one. The high seroprevalence of dengue virus infection in Jazan region indicates its endemicity. The present study highlights the importance of tracking the spread of dengue virus types and its implication for analyzing changes in dengue endemicity in specified areas over time. Continuous surveillance of dengue virus serotypes in the region to detect as earlier the local origin circulating serotypes from the imported ones especially new types DEN-4 and DEN-5, for which continued surveillance is imperative.

Recommendations

Dengue virus (DENV) is a mosquito-borne flavivirus found in the tropical and subtropical regions causing a substantial economic and disease burden. The main vector, *Aedes aegypti*, thrives in urban areas across these regions, although rural areas are increasingly affected. WHO estimated that over 50 million DENV infections occurred annually?

The rapid and dependable diagnosis of the suspected dengue patient among intruders or travelers is a must

The control of the mosquito-vector is a must. One must take into consideration that the infected female *Aedes* species passes the virus by transovarian. Thus, the immature stages as well as the adults must be eradicated.

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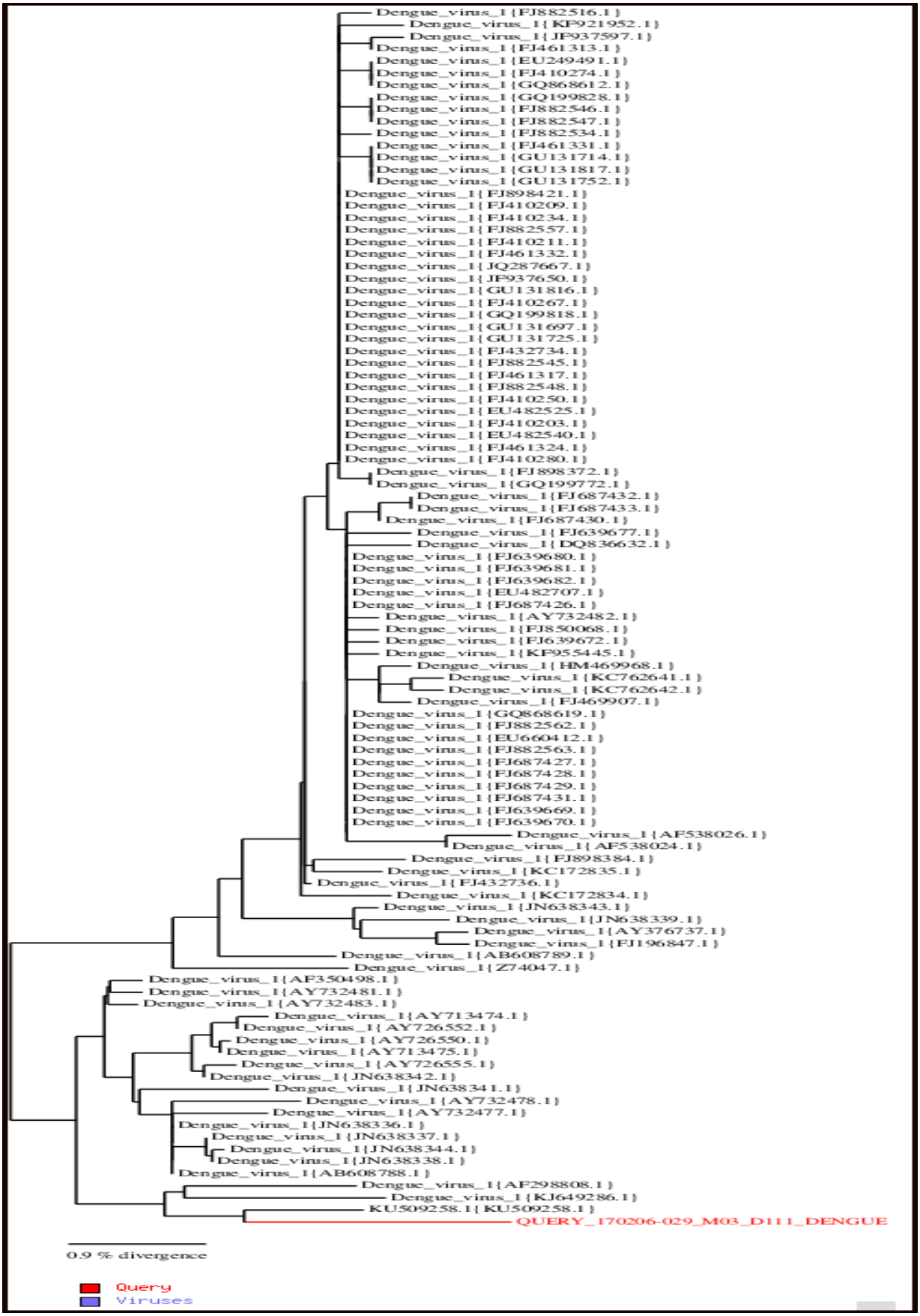

Fig.6. DEN-1 serotype similarity tree.

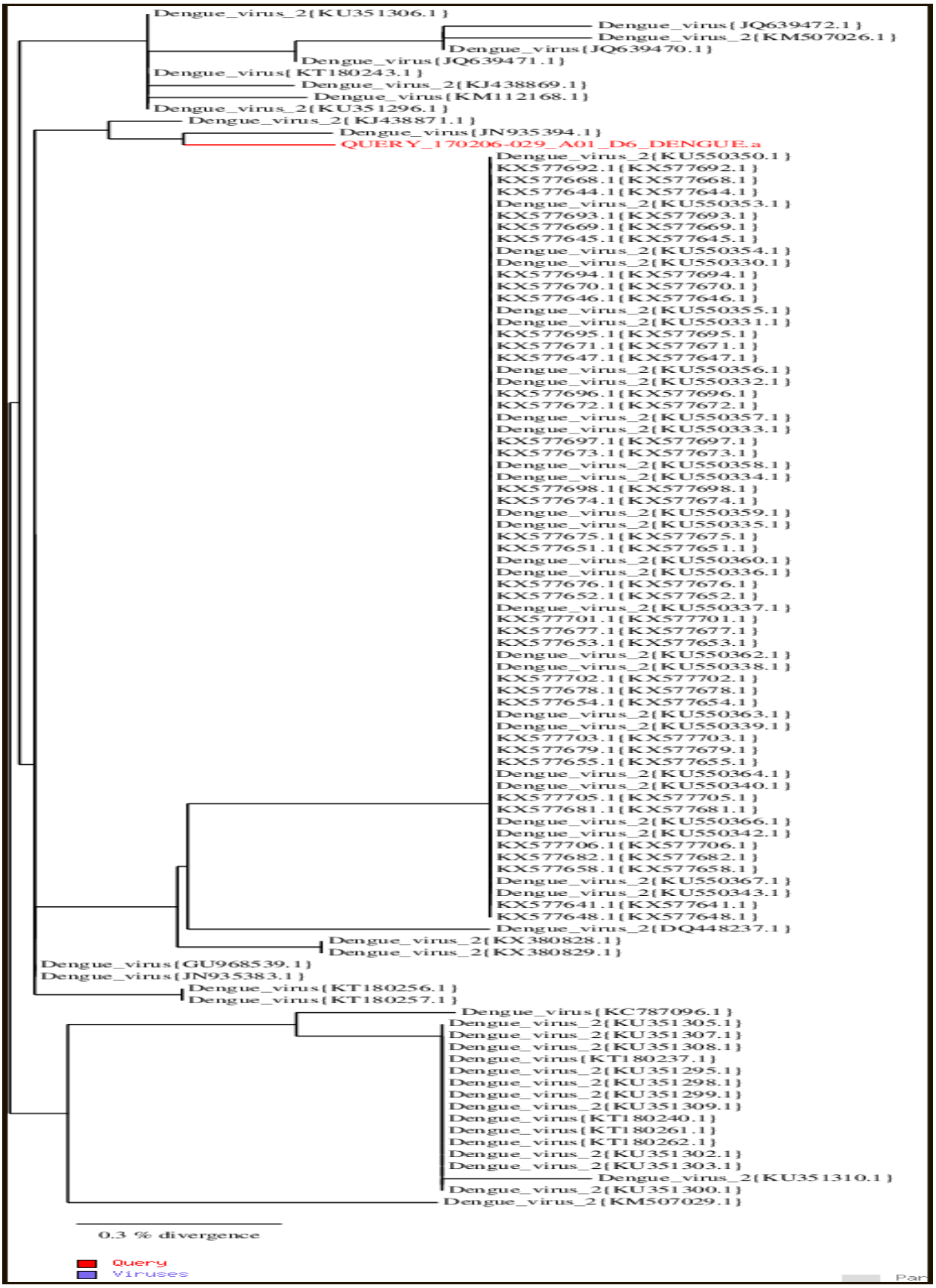

Fig.7. DEN-2 serotype similarity tree.

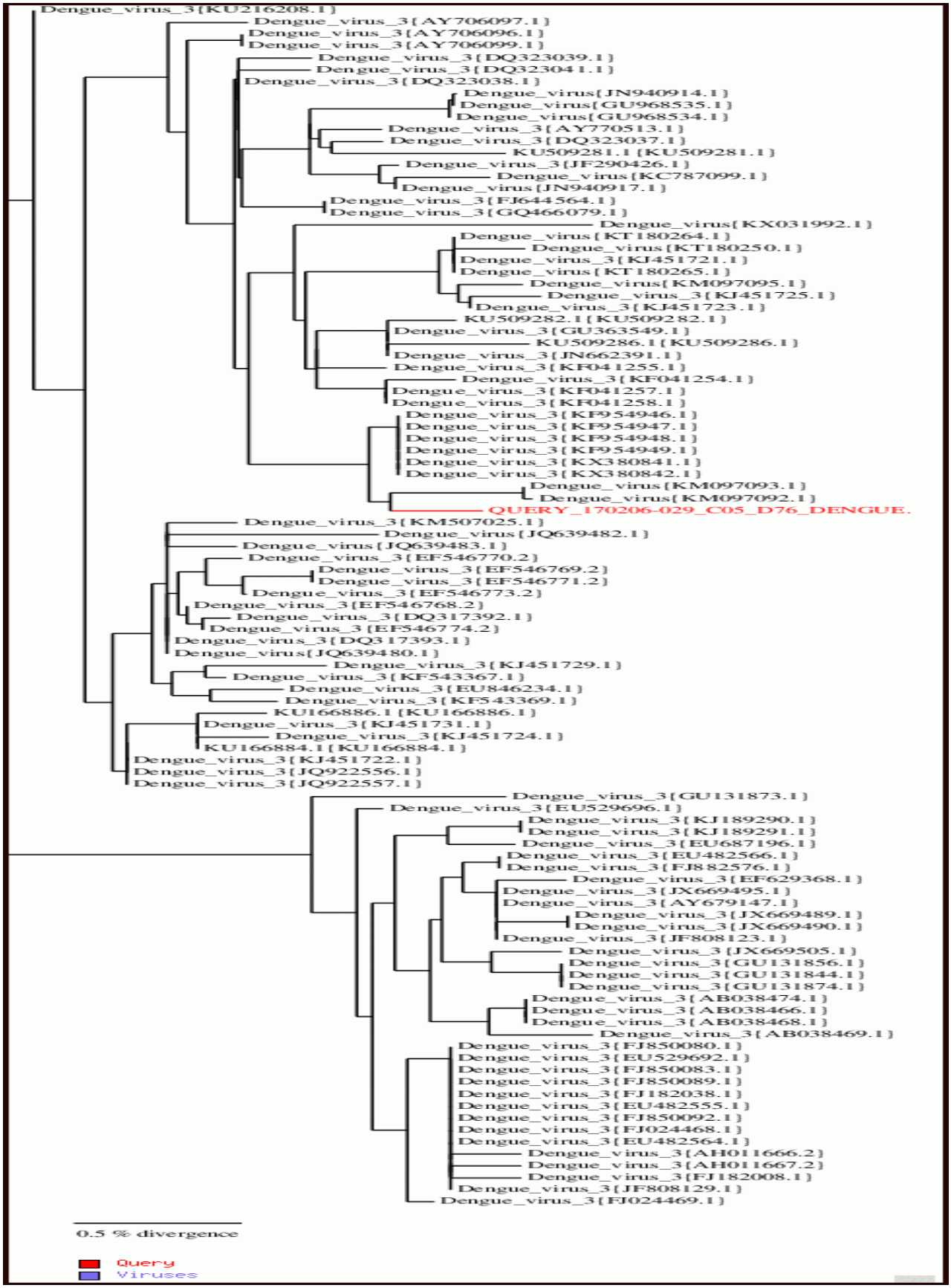

Fig. 8: DEN-3 serotype similarity tree.