

# Host-Range shift between emerging P[8]-4 Rotavirus and Common P[8] and P[4] Strains

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### 1 HOST-RANGE SHIFT BETWEEN EMERGING P[8]-4 ROTAVIRUS AND

## 2 COMMON P[8] and P[4] STRAINS

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- 4 Marie Imbert-Marcille<sup>2,3</sup>, Nathalie Ruvoen-Clouet<sup>1,4</sup>, Mahjoub Aouni<sup>5</sup>, Maha Mastouri<sup>5</sup>,
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- 22 individuals, preferentially infecting the same individuals. By contrast, co-circulating and
- 23 recently emerged P[8]-4 strains attach to saliva regardless of the secretor status and infect
- 24 preferentially nonsecretor children.

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# 42 Abstract

43	In Tunisia, we observed that rotavirus P[8]-3 and P[4] in young children with gastroenteritis
44	associate with the secretor histo-blood-group phenotype. Inversely, the emerging P[8]-4,
45	representing 10% of cases, was exclusively found among nonsecretor patients. Unlike VP8*
46	from P[8]-3 and P[4] strains, the P[8]-4 VP8* protein attached to glycans from saliva samples
47	regardless of the donors Secretor status. Interestingly, a high frequency of FUT2 enzyme
48	deficiency (nonsecretor phenotype) was observed in the population. This may allow co-
49	circulation of P[8]-3 and P[8]-4 strains in secretor and nonsecretor children, respectively.
50	
51	Keywords: rotavirus, histo-blood group antigen, secretor/nonsecretor phenotypes,
52	fucosyltransferase, FUT2, genetic susceptibility, host-range

#### 55 Background

Rotavirus remains the leading cause of severe diarrheal disease in children under 5 globally 56 despite a remarkable decrease in the yearly number of associated children deaths since 57 58 vaccine introduction [1]. Unlike in high-income countries, vaccine efficacy appears limited in 59 many low-income areas, reasons for this likely being multifactorial although still ill-defined [2]. At the initiation of infection, human rotavirus strains bind to glycans of the histo-blood 60 group antigens type (HBGAs) through the VP4 capsid spike protein that define strains P types 61 62 [3]. HBGAs are expressed in the gut mucosa, mainly as O-glycans from soluble or cell surface bound mucins and as membrane glycolipids. P[8] strains that account for 74% of the 63 global prevalence of VP4 [4] attach to fucosylated glycans dependent on the FUT2 64 fucosyltransferase for synthesis. As a result, children of the said nonsecretor phenotype who 65 66 lack a functional FUT2 allele appear to be much more resistant to the disease than the socalled secretor children who possess at least one functional allele, thereby expressing  $\alpha 1, 2$ -67 linked fucose residues in their small intestinal mucosa [5]. Although the strong protective 68 effect of the nonsecretor phenotype was observed in various countries from Europe, North 69 70 America and Africa, it could not be detected in either Tunisia or Bangladesh [6, 7]. Here we 71 conducted a new study in Tunisia to understand those between-studies or geographical discrepancies. 72

#### 74 Methods

#### 75 **Patients and control samples:**

Fecal, serum and saliva samples were collected between November 2015 and October 2018 76 from 189 young children in Tunisia. Only 42 saliva samples were obtained and used to 77 78 control for the match with the genotype performed on serum samples. The specimens were collected from infants and young children between 20 days and 4.5 years of age presenting 79 80 with acute gastroenteritis to the hospital Fattouma Bourguiba (Monastir). Saliva samples 81 from a control population composed of 103 adults coming from different regions were also 82 collected in order to obtain HBGA phenotypes and genotypes frequencies in Tunisia. The study was approved by the Ethics Committee of the Fattouma Bourguiba University Hospital 83 84 in Monastir.

#### 85 Virus detection and P type genotyping:

Nucleic acids were extracted from approximately 100 mg stool samples carried by an 86 automatic extractor type IpreP, using the manufacturer's iPrep<sup>™</sup> PureLink<sup>™</sup> Virus Kit 87 (Invitrogen). Rotavirus was diagnosed by real-time multiplex PCR FTD Viral gastroenteritis 88 (Fast-track diagnostics) allowing the detection of enteric viruses. An 850 bp fragment of 89 90 VP8\* sequences was obtained from a subset of 63 cases using consensus primers [8]. 91 Sequences accession numbers are given in the Supplementary data. These sequences allowed 92 classification of circulating strains at the P-type level. When Ct values were <18-20 a one-93 step RT-PCR was performed using the Takara's one step PrimeScript RT-qPCR with 5µL 94 nucleic acids extract and ROTA CON3 M13F / ROTA CON2 M13R primers. When Ct values 95 were >18-20, a first RT-PCR was performed using ROTA CON3F / ROTA CON2R and between 5-10 µL nucleic acids extract, followed by a nested PCR with ROTA VP4 M13F / ROTA VP4 M13R 96 97 primers and the Takara's Premix Ex Taq kit. In cases no amplification was obtained, a first RT-PCR

- 98 was performed with ROTA VP4F / ROTA VP4R primers followed by the nested PCR as above.
- 99 Primers sequences are given in the Supplementary data.

### 100 Determination of the Secretor status and saliva OSGE treatment:

- 101 Patients serum samples were used to extract genomic DNA for FUT2 genotyping as
- 102 previously described and the secretor/nonsecretor phenotype was deduced from the genotype
- 103 [9]. Saliva samples obtained from a control Tunisian population composed of 103 adults were
- 104 used for HBGA phenotyping and genotyping as previously described [10] in order to obtain
- 105 HBGA phenotypes frequencies. Genotyping was used for validation of the phenotypes.
- 106 To ascertain attachment to O-glycans, salivary mucins were diluted 1 in 250 in PBS and
- 107 incubated or not with 12 μl of O-Sialoglycoprotein Endopeptidase (1,2 mg/ml;
- 108 CEDARLANE, Burlington, Canada) in a water bath at 37°C overnight. After a 1 in 4 dilution
- 109 in coating buffer pH 9,5 (Pierce, Thermo Fisher Scientific), 100 µl of the treated mucins were
- 110 dispatched in each well of a Maxisorp 96-well plate (Nunc) which was then incubated
- 111 overnight at 4°C. The plate was washed 3 times with PBS-0,05% Tween20, blocked with
- 112 PBS-5% BSA for several hours at 37°C and ELISA was performed with VP8\* proteins as
- 113 previoulsy described [11].

114

#### 116 Results

117 All rotavirus cases (n=115) were infected by P[8] or P[4] strains. Rotavirus was the single viral agent detected in 45 (39%) cases, whilst co-infection with either astrovirus, adenovirus, 118 119 norovirus and/or sapovirus was found in the remaining 70 (61%) cases. Construction of a 120 phylogenetic tree from the 63 available sequences revealed that 48 sequences (76%) were of 121 the P[8]-3 subtype, 9 (14%) were of the P[4] genotype and 6 (10%) were of the P[8]-4 subtype (Fig. 1S). The latter strains, initially termed OP-354-like, correspond to an antigenic 122 123 variant also called P[8]b contrasting with P[8]a strains [12, 13]. The Secretor phenotype (or FUT2 genotype) could be unambiguously obtained for 114 RVA+ 124 125 patients. In comparison with the adult control group where the proportion of nonsecretors was 126 high (36% as compared with <20% among people of European origin), a lower frequency of nonsecretors was found among rotavirus-infected patients (20% vs 36%, p<0.01). Analysis of 127 128 the patients group without co-infection showed no significant effect of the FUT2 status, likely owing to the small number of cases. Splitting cases between those infected with P[4], P[8]-3 129 versus those infected by P[8]-4 revealed a clear-cut demarcation since all P[8]-4 were found 130 among nonsecretors, whilst all P[4] were found among secretors and P[8]-3 were also 131 132 preferentially found among secretors (Table 1). 133 Previous studies documented binding of the VP8\* domain from P[8] and P[4] strains to  $\alpha$ 1,2fucosylated glycans [3]. Our group showed attachment of the VP8\* from recent P[8]-3 134

135 clinical as well as the P[8]-1 and P[8]-2 vaccine strains to salivary mucins from secretor

136 individuals only [11]. In order to relate the above described epidemiological profile to HBGA

137 binding, VP8\*-GST fusion proteins from a P[4] (TU35) and a P[8]-4 strain (TU7) were

138 produced as previously described in order to assay by ELISA their binding to saliva samples

139 from previously HBGA-phenotyped individuals [11]. As shown on Fig. 1a, binding of the

P[4] VP8\* was limited to saliva from secretor donors. By contrast, P[8]-4 VP8\* attached to
saliva regardless of the Secretor character (Fig. 1b). To ascertain that binding occurred to Olinked glycans, a saliva sample was pretreated by the enzyme O-sialoglycoprotein
endopeptidase (OSGE) that cleaves O-sialoglycoproteins but not unglycosylated or Nglycosylated proteins. We observed that binding to saliva of both the P[4] and the P[8]-4
VP8\* was strongly diminished following OSGE treatment, indicating similar O-glycan
dependence in the saliva binding assay (Fig. 1c).

#### 147 **Discussion**

Here we confirm that commonly circulating P[8]-3 strains cause gastroenteritis in children of 148 149 the secretor phenotype preferentially, albeit not exclusively since a fraction of nonsecretors 150 also appeared among patients. We also showed that P[4] strains infected secretors only. At 151 variance, the emergent P[8]-4 strain was found among nonsecretors but not among secretor 152 children. As the number of patients with P[4] and P[8]-4 was limited, it can be stated that 153 P[8]-4 preferentially infects nonsecretors, whilst other P[8] strains, as well as P[4] 154 preferentially infect secretors. This confirms that, by mediating virus attachment, HBGAs are 155 important host factors determining susceptibility to rotavirus gastroenteritis, but that 156 depending on the circulating strains distinct subgroups in the population may be at higher 157 risk.

The VP8\* fragment of the P[8]-4 VP4 protein attached equally well to saliva from secretors and nonsecretors, indicating a change in carbohydrate specificity in comparison with all other P[8] strains (P[8]-1, P[8]-2, P[8]-3) that bind exclusively to saliva from secretors [11]. The precise carbohydrate motif recognized by the VP8\* of P[8]-4 strains remains to be characterized but it is unlikely to involve an  $\alpha$ 1,2-linked fucose as we failed to observe binding to various synthetic oligosaccharides containing this motif (data not shown). The

reason why P[8]-4 was detected in nonsecretors only is unclear but may be due to a
competition with the more frequently encountered P[8]-3 and P[4] that largely spare
nonsecretors.

167	Zeller et al indicated in 2015 that P[8]-4 originated in Asia and can be considered endemic in
168	Bangladesh, unlike in Sub-Saharan Africa, Europe and North America [13]. This was
169	confirmed by a recent study where a large fraction of cases in Bangladesh were reported to be
170	due to P[8]-4 rotavirus [7]. An earlier study showed that all P[8] rotavirus circulating in
171	Tunisia between 2006 and 2011 were of the P[8]-3 subtype [14]. Similar to our present
172	observation in Tunisia, a recent report detected 10% P[8]-4 in Ghana, suggesting a recent
173	emergence of P[8]-4 in both Northern and Sub-Saharan Africa. Alternatively, in earlier
174	studies P[8]-4 strains may have been undetected due to primers mismatches [15].
175	Interestingly, the nonsecretor phenotype appears at high frequency both in Bangladesh [7] and
176	Tunisia. This may allow P[8]-4 viruses to occupy a host niche of sufficiently large size to
177	maintain transmission in the context of competition with P[8]-3 strains.
178	In accordance with the fact that the commonly used vaccines Rotarix and RotaTeq are
179	attenuated live vaccines based on P[8] strains that require an $\alpha$ 1,2-linked fucose residue for
180	attachment, recent studies reported reduced vaccine efficacy and seroconvertion in
181	nonsecretor children in comparison with secretors [5, 7]. Considering that P[8]-4 strains
182	represent antigenic variants that readily infect nonsecretor children, the presence of such
183	variants may have a significant impact on vaccine efficacy in populations where HBGA
184	polymorphisms allows their circulation at high frequency.

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### 191 **References**

- 192 1. Troeger C, Khalil IA, Rao PC, et al. Rotavirus Vaccination and the Global Burden of Rotavirus
- 193 Diarrhea Among Children Younger Than 5 Years. JAMA Pediatr **2018**; 172:958-65.
- 194 2. Glass RI, Parashar U, Patel M, Gentsch J, Jiang B. Rotavirus vaccines: successes and challenges.
- 195 The Journal of infection **2014**; 68 Suppl 1:18.
- 196 3. Tan M, Jiang X. Histo-blood group antigens: a common niche for norovirus and rotavirus.
- 197 Expert Rev Mol Med **2014**; 16:e5.
- 198 4. Banyai K, Laszlo B, Steele AD, Nelson EA, Gentsch JR, Parashar UD. Systematic review of
- 199 regional and temporal trends in global rotavirus strain diversity in the pre rotavirus vaccine era:
- insights for understanding the impact of rotavirus vaccination programs. Vaccine 2012; 30
   Suppl 1:A:122-30.
- 202 5. Ramani S, Giri S. Influence of histo blood group antigen expression on susceptibility to enteric
  203 viruses and vaccines. Cur Op Infect Dis 2019; 32:445-52.
- 204 6. Ayouni S, Sdiri-Loulizi K, de Rougemont A, et al. Rotavirus P[8] Infections in Persons with
- 205 Secretor and Nonsecretor Phenotypes, Tunisia. Emerg Infect Dis **2015**; 21:2055-8.
- 206 7. Lee B, Dickson DM, deCamp AC, et al. Histo-Blood Group Antigen Phenotype Determines
- 207 Susceptibility to Genotype-Specific Rotavirus Infections and Impacts Measures of Rotavirus
- 208 Vaccine Efficacy. J Infect Dis **2018**; 217:1399-407.
- 8. Imbert-Marcille B-M, Barbé L, Dupé M, et al. A FUT2 gene common polymorphism determines
  resistance to rotavirus A of the P[8] genotype. J Infect Dis 2013; 209:1227-30.
- 9. Hutson AM, Airaud F, Le Pendu J, Estes MK, Atmar RL. Norwalk virus infection associates with
- secretor status genotyped from sera. J Med Virol **2005**; 77:116-20.
- 213 10. Marionneau S, Airaud F, Bovin NV, Le Pendu J, Ruvoën-Clouet N. Influence of the combined
- ABO, FUT2 and FUT3 polymorphism on susceptibility to Norwalk virus attachment. J Infect Dis **2005**: 192:1071-7.
- 216 11. Barbé L, Le Moullac-Vaidye B, Echasserieau K, et al. Histo-blood group antigen-binding
- specificities of human rotaviruses are associated with gastroenteritis but not with in vitro
   infection. Scientific Reports **2018**; 8:12961.
- 219 12. Nagashima S, Kobayashi N, Paul SK, Alam MM, Chawla-Sarkar M, Krishnan T.
- Characterization of full-length VP4 genes of OP354-like P[8] human rotavirus strains detected in
   Bangladesh representing a novel P[8] subtype. Arch Virol **2009**; 154:1223-31.
- 222 13. Zeller M, Heylen E, Damanka S, et al. Emerging OP354-Like P[8] Rotaviruses Have Rapidly
- Dispersed from Asia to Other Continents. Mol Biol Evol **2015**; 32:2060-71.
- 224 14. Ben Hadj Fredj M, BenHamida-Rebaï M, Heylen E, et al. Sequence and phylogenetic analyses
- of human rotavirus strains: Comparison of VP7 and VP8/ antigenic epitopes between Tunisian
- and vaccine strains before national rotavirus vaccine introduction. Infect Genet Evol 2013;
   18:132-44.
- 15. Damanka SA, Agbemabiese CA, Dennis FE, et al. Genetic analysis of Ghanaian G1P[8] and G9P
- [8] rotavirus A strains reveals the impact of P[8] VP4 gene polymorphism on P-genotyping. PLoS
- 230 One **2019**; 14:e0218790.
- 231

## 233 Table 1

	Controls		All RVA+ (%)		F	RVA+ only (%	)
	(%)						
secretors	66 (64)		89 (80)**			32 (74) <sup>n.s.</sup>	
nonsecretors	37 (36)		22 (20)			11 (26)	
		P[4]	P[8]-3	P[8]-4	P[4]	P[8]-3	P[8]-4
secretors		7 (100)***	39 (85)****	0 (0)	2 (100) <sup>n.s.</sup>	20 (90)**	0 (0)
300101013			()	- (-)	2 (100)	()	- (-)
nonsecretors		0 (0)	7 (15)	6 (100)	0 (0)	2 (9)	4 (0)

#### Associations between the Secretor type and rotavirus positivity

235 Upper part: Comparisons between the group of controls and groups of RVA-infected patients: all

236 RVA+ cases or RVA+ without co-infection (RVA+ only). Lower part: Comparisons between either

the P[4] or the P[8]-3 infected groups versus the P[8]-4 infected group were performed by 2-tailed

238 Fisher's exact test. \*\*\*\* p<0.0001, \*\*\* p<0.001, \*\* p<0.01, \* p<0.05, n.s. p>0.05.

239





#### 242 Figure Legend

VP8\* saliva binding. Comparison of binding of P[4]VP8\* (TU35) (a) and P[8]-4 VP8\* (TU7) 243 (b) to saliva from secretors (n=43) and nonsecretors (n=14) tested by ELISA on immobilized 244 245 saliva samples. These saliva samples were from adults and previously described [11] Values +/- SEM are shown and significance levels based on Welch's t-test. \*\*\*\*p<0.0001, n.s. 246 p>0.05. Effect of the sialoglycoprotein endopeptidase treatment on binding of P[4] (TU35) 247 and P[8]-4 (TU7) VP8\* to the saliva sample from a secretor and Lewis positive individual. 248 Saliva diluted 1/250 was treated with 12 µL of enzyme (+) or enzyme buffer only (-) 249 overnight at 37°C prior coating at a further <sup>1</sup>/<sub>4</sub> dilution onto ELISA plates. Results represent 250 251 the mean +/- SEM from three independent experiments performed in duplicates (c). 252

253

# 255 Supplementary data

# 256 GenBank accession numbers of VP8\* coding sequences from Tunisian cases included in

## 257 the phylogenetic tree (Fig. 1S).

259         TU187         MN836721         Sec           260         TU219         MN836722         Sec           261         TU55         MN836724         Sec           262         TU138         MN836724         Sec           263         TU173         MN836725         nonsec           264         TU93         MN836726         Sec           265         TU132         MN836728         Sec           266         TU123         MN836729         Sec           267         TU83         MN836730         Sec           269         TU91         MN836731         Sec           270         TU63         MN836733         Sec           271         TU33         MN836736         nonsec           273         TU008         MN836737         Sec           274         TU31         MN836738         Sec           275         TU84         MN836737         Sec           276         TU37         MN836740         Sec           277         TU29         MN836741         Sec           280         TU118         MN836744         Sec           281         TU44         MN83	258	Case identification	GenBank number	Patient's phenotype <sup>a</sup>
260         TU219         MN836722         Sec           261         TU55         MN836723         Sec           262         TU138         MN836724         Sec           263         TU173         MN836726         Sec           264         TU33         MN836726         Sec           265         TU132         MN836727         Sec           266         TU200         MN836729         Sec           267         TU83         MN836731         Sec           268         TU200         MN836732         Sec           270         TU63         MN836733         Sec           271         TU33         MN836735         nonsec           271         TU33         MN836736         Sec           273         TU008         MN836737         Sec           274         TU131         MN836738         Sec           275         TU84         MN836740         Sec           276         TU37         MN836741         Sec           278         TU52         MN836743         ?           281         TU84         MN836743         ?           282         TU44         MN836743 <td>259</td> <td>TU187</td> <td>MN836721</td> <td>Sec</td>	259	TU187	MN836721	Sec
253         70.7         70.8         80.2           261         TU55         MN836723         Sec           263         TU173         MN836725         nonsec           264         TU93         MN836726         Sec           265         TU122         MN836728         Sec           266         TU123         MN836728         Sec           267         TU83         MN836731         Sec           268         TU200         MN836731         Sec           270         TU63         MN836732         Sec           271         TU33         MN836734         nonsec           271         TU33         MN836735         nonsec           271         TU33         MN836736         Sec           272         TU232         MN836737         Sec           273         TU008         MN836737         Sec           274         TU31         MN836738         Sec           275         TU84         MN836740         Sec           279         TU76         MN836743         ?           281         TU81         MN836743         ?           282         TU44         MN836744 </td <td>260</td> <td>TU219</td> <td>MN836722</td> <td>Sec</td>	260	TU219	MN836722	Sec
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202         1013         MIN836725         nonsec           264         TU133         MIN836726         Sec           265         TU132         MIN836728         Sec           266         TU20         MIN836728         Sec           267         TU83         MIN836731         Sec           268         TU200         MIN836731         Sec           270         TU63         MIN836734         nonsec           271         TU33         MIN836735         nonsec           271         TU33         MIN836736         Sec           272         TU222         MIN836736         Sec           273         TU008         MIN836738         Sec           274         TU131         MIN836738         Sec           275         TU84         MIN836741         Sec           279         TU52         MIN836741         Sec           280         TU118         MIN836743         ?           281         TU44         MIN836744         Sec           283         TU98         MIN836744         Sec           284         TU263         MIN836748         Sec           285         TU176 </td <td>262</td> <td>TU138</td> <td>MN836724</td> <td>Sec</td>	262	TU138	MN836724	Sec
263         1013         MIN36726         Sec           264         1033         MIN36726         Sec           265         TU132         MIN36728         Sec           266         TU123         MIN36729         Sec           268         TU200         MIN366730         Sec           269         TU91         MIN36731         Sec           270         TU63         MIN36732         Sec           271         TU33         MIN36732         Sec           271         TU33         MIN36735         nonsec           273         TU008         MIN36735         nonsec           274         TU131         MIN36738         Sec           275         TU84         MIN36738         Sec           276         TU37         MIN36738         Sec           277         TU29         MIN36742         Sec           280         TU118         MIN36744         Sec           281         TU44         MIN36745         nonsec           284         TU28         MIN36748         Sec           285         TU176         MIN36751         Sec           286         TU249	263	TU173	MN836725	nonsec
201         TU32         MN836727         Sec           265         TU132         MN836728         Sec           266         TU23         MN836729         Sec           268         TU200         MN836730         Sec           269         TU91         MN836732         Sec           270         TU63         MN836733         Sec           271         TU33         MN836734         nonsec           273         TU008         MN836737         Sec           274         TU131         MN836737         Sec           275         TU84         MN836737         Sec           276         TU37         MN836740         Sec           277         TU29         MN836740         Sec           279         TU76         MN836741         Sec           280         TU118         MN836743         ?           281         TU81         MN836744         Sec           283         TU98         MN836747         Sec           284         TU263         MN836747         Sec           285         TU176         MN836747         Sec           286         TU249         MN836750<	264	TU 193	MN836726	Sec
205         10122         MINB36728         Sec           266         TU123         MINB36728         Sec           268         TU200         MINB36730         Sec           269         TU91         MINB36731         Sec           270         TU63         MINB36731         Sec           271         TU33         MINB36735         nonsec           272         TU232         MINB36735         nonsec           274         TU131         MINB36736         Sec           275         TU84         MINB36738         Sec           276         TU37         MINB36738         Sec           277         TU29         MINB36741         Sec           276         TU37         MINB36742         Sec           280         TU118         MINB36743         ?           281         TU81         MINB36744         Sec           283         TU98         MINB36748         Sec           284         TU263         MINB36748         Sec           285         TU176         MINB36749         Sec           286         TU42         MINB36749         Sec           287         TU42	265	TU132	MN836727	Sec
203         TUB3         MN836729         Sec           268         TU200         MN836730         Sec           269         TU91         MN836731         Sec           270         TU63         MN836732         Sec           271         TU33         MN836733         Sec           272         TU232         MN836736         Sec           273         TU008         MN836736         Sec           274         TU131         MN836736         Sec           275         TU84         MN836739         Sec           276         TU37         MN836740         Sec           277         TU29         MN836741         Sec           278         TU52         MN836743         ?           280         TU118         MN836743         ?           281         TU84         MN836746         Sec           283         TU98         MN836746         Sec           284         TU263         MN836748         Sec           285         TU176         MN836750         Sec           286         TU264         MN836753         nonsec           287         TU42         MN836753 <td>266</td> <td>TU123</td> <td>MN836728</td> <td>Sec</td>	266	TU123	MN836728	Sec
Construction         Construction         Construction           C68         TU200         MN836730         Sec           C70         TU63         MN836731         Sec           C71         TU33         MN836732         Sec           C71         TU33         MN836733         Sec           C72         TU232         MN836735         nonsec           C73         TU008         MN836736         Sec           C74         TU131         MN836737         Sec           C75         TU84         MN836737         Sec           C76         TU37         MN836738         Sec           C77         TU29         MN836741         Sec           C79         TU52         MN836741         Sec           C80         TU118         MN836745         nonsec           C81         TU83         MN836746         Sec           C83         TU44         MN836746         Sec           C84         TU263         MN836748         Sec           C85         TU176         MN836746         Sec           C86         TU263         MN836751         Sec           C86         TU264         MN8	267	TU83	MN836729	Sec
269         TUS1         MN836731         Sec           270         TU63         MN836731         Sec           271         TU33         MN836733         Sec           272         TU232         MN836735         nonsec           273         TU008         MN836735         nonsec           274         TU131         MN836736         Sec           275         TU84         MN836738         Sec           276         TU37         MN836738         Sec           276         TU37         MN836738         Sec           276         TU37         MN836740         Sec           277         TU29         MN836741         Sec           280         TU118         MN836742         Sec           281         TU81         MN836743         ?           282         TU44         MN836745         nonsec           284         TU263         MN836746         Sec           285         TU176         MN836747         Sec           286         TU249         MN836751         Sec           287         TU42         MN836751         Sec           289         TU48         MN83	268	TU200	MN836730	Sec
270       TU63       MN836732       Sec         271       TU33       MN836732       Sec         271       TU33       MN836733       Sec         273       TU008       MN836735       nonsec         273       TU008       MN836736       Sec         274       TU131       MN836736       Sec         275       TU84       MN836737       Sec         276       TU37       MN836738       Sec         277       TU29       MN836740       Sec         279       TU76       MN836741       Sec         280       TU118       MN836743       ?         281       TU81       MN836746       Sec         283       TU98       MN836746       Sec         284       TU263       MN836746       Sec         285       TU176       MN836747       Sec         286       TU249       MN836750       Sec         287       TU42       MN836751       Sec         289       TU90       MN836753       nonsec         289       TU90       MN836753       sec         290       TU26       MN836753       sec	269	TU91	MN836731	Sec
271       TU33       MN836732       Sec         272       TU33       MN836733       Sec         273       TU008       MN836735       nonsec         274       TU131       MN836736       Sec         275       TU84       MN836737       Sec         276       TU37       MN836738       Sec         277       TU29       MN836739       Sec         278       TU52       MN836741       Sec         279       TU76       MN836743       ?         280       TU18       MN836745       nonsec         281       TU81       MN836744       Sec         283       TU98       MN836745       nonsec         284       TU263       MN836748       Sec         285       TU176       MN836748       Sec         286       TU18       MN836753       nonsec         287       TU42       MN836753       nonsec         288       TU108       MN836753       nonsec         290       TU26       MN836753       nonsec         291       TU286       MN836755       Sec         293       TU48       MN836758       Sec	270	TU63	MN836732	Sec
2712       TU232       MN836734       nonsec         273       TU008       MN836735       nonsec         274       TU131       MN836736       Sec         275       TU84       MN836737       Sec         276       TU37       MN836738       Sec         2776       TU29       MN836739       Sec         2777       TU29       MN836740       Sec         278       TU52       MN836741       Sec         280       TU118       MN836743       ?         281       TU81       MN836743       ?         282       TU44       MN836744       Sec         283       TU98       MN836746       Sec         284       TU263       MN836747       Sec         285       TU176       MN836748       Sec         286       TU263       MN836750       Sec         287       TU42       MN836751       Sec         288       TU108       MN836752       Sec         290       TU26       MN836753       nonsec         291       TU286       MN836756       Sec         293       TU18       MN836757       nonsec	270	TU33	MN836733	Sec
273       TU022       MN030734       Informed         273       TU008       MN836735       nonsec         274       TU131       MN836736       Sec         275       TU84       MN836737       Sec         276       TU37       MN836738       Sec         277       TU29       MN836740       Sec         278       TU52       MN836741       Sec         280       TU118       MN836744       Sec         281       TU81       MN836744       Sec         283       TU98       MN836746       Sec         284       TU263       MN836746       Sec         285       TU176       MN836747       Sec         286       TU249       MN836748       Sec         287       TU42       MN836750       Sec         288       TU108       MN836751       Sec         290       TU26       MN836753       nonsec         291       TU286       MN836755       Sec         293       TU18       MN836755       Sec         294       TU48       MN836756       Sec         295       TU69       MN836757       nonsec	271	TU232	MN836734	nonsec
274       TU131       MN836736       Sec         274       TU131       MN836736       Sec         275       TU84       MN836737       Sec         276       TU37       MN836738       Sec         277       TU29       MN836740       Sec         278       TU52       MN836741       Sec         279       TU76       MN836742       Sec         280       TU118       MN836744       Sec         281       TU81       MN836746       Sec         283       TU98       MN836746       Sec         284       TU263       MN836747       Sec         285       TU176       MN836748       Sec         286       TU249       MN836750       Sec         287       TU42       MN836751       Sec         288       TU108       MN836752       Sec         290       TU26       MN836753       nonsec         291       TU286       MN836755       Sec         293       TU18       MN836756       Sec         294       TU48       MN836758       Sec         295       TU69       MN836758       Sec	272	TU008	MN836735	nonsec
274       10101       MN830730       Sec         275       TU84       MN836737       Sec         276       TU37       MN836738       Sec         277       TU29       MN836739       Sec         278       TU52       MN836740       Sec         279       TU76       MN836741       Sec         280       TU118       MN836743       ?         281       TU81       MN836744       Sec         283       TU98       MN836745       nonsec         284       TU263       MN836746       Sec         285       TU176       MN836747       Sec         286       TU249       MN836748       Sec         287       TU42       MN836750       Sec         288       TU108       MN836751       Sec         289       TU90       MN836753       nonsec         290       TU26       MN836753       nonsec         291       TU86       MN836755       Sec         293       TU18       MN836756       Sec         294       TU48       MN836758       Sec         295       TU69       MN836759       Sec	273	TU121	MN836736	Soc
27.6       TU37       Mindsorof       Sec         27.7       TU29       Mindsorof       Sec         27.8       TU52       Mindsorof       Sec         27.8       TU52       Mindsorof       Sec         27.8       TU52       Mindsorof       Sec         27.9       TU76       Mindsorof       Sec         280       TU118       Mindsorof       Sec         281       TU81       Mindsorof       Sec         283       TU98       Mindsorof       Sec         284       TU263       Mindsorof       Sec         285       TU176       Mindsorof       Sec         286       TU249       Mindsorof       Sec         287       TU42       Mindsorof       Sec         288       TU108       Mindsorof       Sec         289       TU90       Mindsorof       Sec         290       TU26       Mindsorof       Sec         291       TU286       Mindsorof       Sec         292       TU9       Mindsorof       Sec         293       TU18       Mindsorof       Sec         294       TU48       Mindsorof       Se	275	TU84	MN836737	Sec
277       TU29       MN836739       Sec         277       TU29       MN836740       Sec         279       TU76       MN836741       Sec         280       TU118       MN836742       Sec         281       TU81       MN836743       ?         282       TU44       MN836744       Sec         283       TU98       MN836745       nonsec         284       TU263       MN836746       Sec         285       TU176       MN836747       Sec         286       TU249       MN836748       Sec         287       TU42       MN836750       Sec         288       TU108       MN836751       Sec         289       TU90       MN836753       nonsec         290       TU26       MN836755       Sec         291       TU286       MN836755       Sec         293       TU18       MN836758       Sec         294       TU48       MN836758       Sec         295       TU69       MN836758       Sec         296       TU58       MN836761       Sec         297       TU49       MN836762       Sec	276	TU37	MN836738	Sec
277       TU25       MN836740       Sec         278       TU26       MN836740       Sec         280       TU18       MN836742       Sec         281       TU81       MN836743       ?         282       TU44       MN836744       Sec         283       TU98       MN836745       nonsec         284       TU263       MN836746       Sec         285       TU176       MN836747       Sec         286       TU249       MN836748       Sec         287       TU42       MN836750       Sec         288       TU108       MN836751       Sec         289       TU26       MN836752       Sec         290       TU26       MN836754       Sec         291       TU286       MN836755       Sec         292       TU9       MN836756       Sec         293       TU18       MN836756       Sec         294       TU48       MN836758       Sec         295       TU69       MN836758       Sec         297       TU170       MN836761       Sec         298       TU49       MN836762       Sec	270	TU20	MN836730	Sec
279       TU76       MN836741       Sec         280       TU118       MN836742       Sec         281       TU81       MN836743       ?         282       TU44       MN836744       Sec         283       TU98       MN836746       Sec         284       TU263       MN836746       Sec         285       TU176       MN836747       Sec         286       TU249       MN836748       Sec         287       TU42       MN836749       Sec         288       TU108       MN836750       Sec         290       TU26       MN836752       Sec         291       TU286       MN836753       nonsec         292       TU9       MN836754       Sec         293       TU18       MN836755       Sec         294       TU48       MN836758       Sec         295       TU69       MN836758       Sec         297       TU170       MN836760       Sec         298       TU49       MN836761       Sec         299       TU283       MN836761       Sec         299       TU283       MN836763       Sec      <	278	TU52	MN836740	Sec
280       TU118       MN836742       Sec         281       TU81       MN836743       ?         282       TU44       MN836743       ?         283       TU98       MN836744       Sec         284       TU263       MN836746       Sec         285       TU176       MN836747       Sec         286       TU249       MN836748       Sec         287       TU42       MN836749       Sec         288       TU108       MN836750       Sec         290       TU26       MN836752       Sec         291       TU286       MN836753       nonsec         292       TU9       MN836756       Sec         293       TU18       MN836756       Sec         294       TU48       MN836758       Sec         295       TU69       MN836758       Sec         295       TU69       MN836760       Sec         297       TU170       MN836761       Sec         298       TU49       MN836762       Sec         299       TU283       MN836763       Sec         300       TU281       MN836763       Sec <tr< td=""><td>279</td><td>TU76</td><td>MN836741</td><td>Sec</td></tr<>	279	TU76	MN836741	Sec
280       TURIC       MIN080742       Sec         281       TU81       MN836743       ?         282       TU44       MN836744       Sec         283       TU98       MN836746       Sec         284       TU263       MN836746       Sec         285       TU176       MN836748       Sec         286       TU249       MN836748       Sec         287       TU42       MN836750       Sec         288       TU108       MN836751       Sec         289       TU90       MN836752       Sec         290       TU26       MN836753       nonsec         291       TU286       MN836754       Sec         292       TU9       MN836755       Sec         293       TU18       MN836756       Sec         294       TU48       MN836758       Sec         295       TU69       MN836758       Sec         296       TU58       MN836760       Sec         297       TU170       MN836761       Sec         298       TU49       MN836763       Sec         300       TU281       MN836763       Sec      <	280	TU118	MN836742	Sec
282       TU44       MN836744       Sec         283       TU98       MN836745       nonsec         284       TU263       MN836746       Sec         285       TU176       MN836748       Sec         286       TU249       MN836749       Sec         287       TU42       MN836750       Sec         288       TU108       MN836752       Sec         289       TU90       MN836753       nonsec         290       TU26       MN836753       nonsec         291       TU286       MN836754       Sec         292       TU9       MN836755       Sec         293       TU18       MN836756       Sec         294       TU48       MN836758       Sec         295       TU69       MN836759       Sec         296       TU58       MN836759       Sec         297       TU170       MN836760       Sec         298       TU49       MN836761       Sec         299       TU283       MN836763       Sec         300       TU281       MN836763       Sec         301       TU15       MN836764       Sec <td>281</td> <td>TU81</td> <td>MN836743</td> <td>2</td>	281	TU81	MN836743	2
283       TU98       MN836745       nonsec         284       TU263       MN836746       Sec         285       TU176       MN836747       Sec         286       TU249       MN836748       Sec         287       TU42       MN836749       Sec         288       TU108       MN836750       Sec         289       TU90       MN836751       Sec         290       TU26       MN836752       Sec         291       TU286       MN836753       nonsec         292       TU9       MN836756       Sec         293       TU18       MN836756       Sec         294       TU48       MN836757       nonsec         295       TU69       MN836759       Sec         296       TU58       MN836760       Sec         297       TU49       MN836761       Sec         298       TU49       MN836763       Sec         299       TU283       MN836763       Sec         300       TU281       MN836764       Sec         301       TU15       MN836765       ?         302       TU147       MN836765       ?	282	TUAA	MN836744	: Sec
284       TU263       MN836746       Sec         285       TU176       MN836747       Sec         286       TU249       MN836748       Sec         287       TU42       MN836749       Sec         288       TU108       MN836750       Sec         289       TU90       MN836751       Sec         290       TU26       MN836752       Sec         291       TU286       MN836753       nonsec         292       TU9       MN836755       Sec         293       TU18       MN836756       Sec         294       TU48       MN836757       nonsec         295       TU69       MN836758       Sec         296       TU58       MN836759       Sec         297       TU170       MN836760       Sec         298       TU49       MN836761       Sec         299       TU283       MN836763       Sec         300       TU281       MN836764       Sec         301       TU15       MN836764       Sec         302       TU147       MN836765       ?         303       TU369       MN836765       ?    <	283	TU 198	MN836745	nonsec
281       TU176       MN836747       Sec         286       TU249       MN836748       Sec         287       TU42       MN836749       Sec         288       TU108       MN836750       Sec         289       TU90       MN836751       Sec         290       TU26       MN836752       Sec         291       TU286       MN836753       nonsec         292       TU9       MN836755       Sec         293       TU18       MN836756       Sec         294       TU48       MN836757       nonsec         295       TU69       MN836758       Sec         297       TU170       MN836759       Sec         298       TU49       MN836760       Sec         299       TU283       MN836761       Sec         299       TU281       MN836763       Sec         300       TU281       MN836763       Sec         301       TU15       MN836764       Sec         302       TU147       MN836765       ?         303       TU369       MN836765       ?	284	TU263	MN836746	Sec
286       TU249       MN836748       Sec         287       TU42       MN836749       Sec         288       TU108       MN836750       Sec         289       TU90       MN836751       Sec         290       TU26       MN836752       Sec         291       TU286       MN836753       nonsec         292       TU9       MN836755       Sec         293       TU18       MN836756       Sec         294       TU48       MN836757       nonsec         295       TU69       MN836758       Sec         296       TU58       MN836759       Sec         297       TU170       MN836760       Sec         298       TU49       MN836761       Sec         299       TU283       MN836762       Sec         300       TU281       MN836763       Sec         301       TU15       MN836764       Sec         302       TU147       MN836765       ?         303       TU369       MN836765       ?	285	TU176	MN836747	Sec
287       TU42       MN836749       Sec         288       TU108       MN836750       Sec         289       TU90       MN836751       Sec         290       TU26       MN836752       Sec         291       TU286       MN836754       Sec         292       TU9       MN836755       Sec         293       TU18       MN836756       Sec         294       TU48       MN836756       Sec         295       TU69       MN836757       nonsec         296       TU58       MN836758       Sec         297       TU170       MN836759       Sec         298       TU49       MN836761       Sec         299       TU283       MN836762       Sec         300       TU281       MN836763       Sec         301       TU15       MN836764       Sec         302       TU147       MN836765       ?         303       TU369       MN836765       ?	286	TI 1249	MN836748	Sec
288       TU108       MN836750       Sec         289       TU90       MN836751       Sec         290       TU26       MN836752       Sec         291       TU286       MN836753       nonsec         292       TU9       MN836755       Sec         293       TU18       MN836756       Sec         294       TU48       MN836756       Sec         295       TU69       MN836757       nonsec         296       TU58       MN836759       Sec         297       TU170       MN836760       Sec         298       TU49       MN836761       Sec         299       TU283       MN836762       Sec         300       TU281       MN836763       Sec         301       TU15       MN836764       Sec         302       TU147       MN836765       ?         303       TU369       MN836765       ?	287	TU42	MN836749	Sec
289       TU90       MN836751       Sec         290       TU26       MN836752       Sec         291       TU286       MN836753       nonsec         292       TU9       MN836754       Sec         293       TU18       MN836755       Sec         294       TU48       MN836756       Sec         295       TU69       MN836757       nonsec         296       TU58       MN836759       Sec         297       TU170       MN836760       Sec         298       TU49       MN836761       Sec         299       TU283       MN836762       Sec         300       TU281       MN836763       Sec         301       TU15       MN836764       Sec         302       TU147       MN836765       ?         303       TU369       MN836765       ?	288	TU108	MN836750	Sec
290       TU26       MN836752       Sec         291       TU286       MN836753       nonsec         292       TU9       MN836754       Sec         293       TU18       MN836755       Sec         294       TU48       MN836756       Sec         295       TU69       MN836757       nonsec         296       TU58       MN836759       Sec         297       TU170       MN836760       Sec         298       TU49       MN836761       Sec         299       TU283       MN836762       Sec         300       TU281       MN836763       Sec         301       TU15       MN836764       Sec         302       TU147       MN836765       ?         303       TU369       MN836765       ?	289	TU90	MN836751	Sec
291       TU286       MN836753       nonsec         292       TU9       MN836754       Sec         293       TU18       MN836755       Sec         294       TU48       MN836756       Sec         295       TU69       MN836757       nonsec         296       TU58       MN836758       Sec         297       TU170       MN836759       Sec         298       TU49       MN836760       Sec         299       TU283       MN836761       Sec         300       TU281       MN836763       Sec         301       TU15       MN836764       Sec         302       TU147       MN836765       ?         303       TU369       MN836765       ?	290	TU26	MN836752	Sec
292       TU9       MN836754       Sec         293       TU18       MN836755       Sec         294       TU48       MN836756       Sec         295       TU69       MN836757       nonsec         296       TU58       MN836759       Sec         297       TU170       MN836759       Sec         298       TU49       MN836760       Sec         299       TU283       MN836761       Sec         300       TU281       MN836763       Sec         301       TU15       MN836764       Sec         302       TU147       MN836765       ?         303       TU369       MN836765       ?	291	TU286	MN836753	nonsec
293       TU18       MN836755       Sec         294       TU48       MN836756       Sec         295       TU69       MN836757       nonsec         296       TU58       MN836759       Sec         297       TU170       MN836759       Sec         298       TU49       MN836760       Sec         299       TU283       MN836761       Sec         300       TU281       MN836762       Sec         301       TU15       MN836764       Sec         302       TU147       MN836765       ?         303       TU369       MN836765       ?	292	TU9	MN836754	Sec
294       TU48       MN836756       Sec         295       TU69       MN836757       nonsec         296       TU58       MN836758       Sec         297       TU170       MN836759       Sec         298       TU49       MN836760       Sec         299       TU283       MN836761       Sec         300       TU281       MN836762       Sec         301       TU15       MN836764       Sec         302       TU147       MN836765       ?         303       TU369       MN836765       ?	293	TU18	MN836755	Sec
295       TU69       MN836757       nonsec         296       TU58       MN836758       Sec         297       TU170       MN836759       Sec         298       TU49       MN836760       Sec         299       TU283       MN836762       Sec         300       TU281       MN836763       Sec         301       TU15       MN836764       Sec         302       TU147       MN836765       ?         303       TU369       MN836765       ?	294	TU48	MN836756	Sec
296       TU58       MN836758       Sec         297       TU170       MN836759       Sec         298       TU49       MN836760       Sec         299       TU283       MN836761       Sec         300       TU281       MN836762       Sec         301       TU15       MN836763       Sec         302       TU147       MN836764       Sec         303       TU369       MN836765       ?	295	TU69	MN836757	nonsec
297       TU170       MN836759       Sec         298       TU49       MN836760       Sec         299       TU283       MN836761       Sec         300       TU281       MN836762       Sec         301       TU15       MN836763       Sec         302       TU147       MN836764       Sec         303       TU369       MN836765       ?	296	TU58	MN836758	Sec
298       TU49       MN836760       Sec         299       TU283       MN836761       Sec         300       TU281       MN836762       Sec         301       TU15       MN836763       Sec         302       TU147       MN836764       Sec         303       TU369       MN836765       ?	297	TU170	MN836759	Sec
299         TU283         MN836761         Sec           300         TU281         MN836762         Sec           301         TU15         MN836763         Sec           302         TU147         MN836764         Sec           303         TU369         MN836765         ?	298	TU49	MN836760	Sec
300         TU281         MN836762         Sec           301         TU15         MN836763         Sec           302         TU147         MN836764         Sec           303         TU369         MN836765         ?	299	TU283	MN836761	Sec
301         TU15         MN836763         Sec           302         TU147         MN836764         Sec           303         TU369         MN836765         ?	300	TU281	MN836762	Sec
302         TU147         MN836764         Sec           303         TU369         MN836765         ?	301	TU15	MN836763	Sec
303 TU369 MN836765 ?	302	TU147	MN836764	Sec
	303	TU369	MN836765	?
304 IU341 MN836766 Sec	304	TU341	MN836766	Sec
305 TU31 MN836767 Sec	305	TU31	MN836767	Sec
306 TU50 MN836768 nonsec	306	TU50	MN836768	nonsec
307 TU7 MN836769 nonsec	307	TU7	MN836769	nonsec
208 TU41 MN096770 PC755	308	TU41	MN836770	nonsec

309	TU115	MN836771	nonsec
310	TU24	MN836772	nonsec
311	TU124	MN836773	nonsec
312	TU102	MN836774	nonsec
313	TU19	MN836775	?
314	TU35	MN836776	Sec
315	TU95	MN836777	Sec
316	TU40	MN836778	Sec
317	TU85	MN836779	Sec
318	TU92	MN836780	Sec
319	TU334	MN836781	Sec
320	TU342	MN836782	Sec
321	TU340	MN836783	?

- 322 <sup>a</sup>Sec= secretor ; nonsec= nonsecretor patient

Potoviruo	nrimoro
Rolavirus	primers

ROTA CON3 M13F	TGTAAAACGACGGCCAGTTGGCTTCGCTCATTTATAGACA
ROTA CON2 M13R	CAGGAAACAGCTATGACCATTTCGGACCATTTATAACC
ROTA VP4 M13F	TGTAAAACGACGGCCAGTTATGCTCCAGTNAATTGG
ROTA VP4 M13R	CAGGAAACAGCTATGACCATTGCATTTCTTTCCATAATG
ROTA CON3F	TGGCTTCGCTCATTTATAGACA
ROTA CON2R	ATTTCGGACCATTTATAACC
ROTA VP4F	TATGCTCCAGTNAATTGG
ROTA VP4R	ATTGCATTTCTTTCCATAATG



Figure 1S. Phylogenetic tree of the VP8\* portion of the VP4 genes in Tunisian strains. The
tree was constructed using the Neighbor Joining method (NJ) and 1000 bootstrap replicates
with the MEGA 7 software. Only bootstrap support values ≥70% are shown. Strains from the
present study are indicated in colored font. The scale bar indicates genetic distances expressed
as nucleotide substitutions per site.