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Sahar Ahmed, Mohamed Abd El-Fatah Mahmoud, Cyril Viarouge, Corinne Sailleau, Stephan Zientara, Emmanuel Breard

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1 PRESENCE OF BLUETONGUE AND EPIZOOTIC HEMORRHAGIC DISEASE VIRUSES  
2 IN EGYPT IN 2016 AND 2017

3 Sahar Ahmed<sup>a</sup>, Mohamed Abd El-Fatah Mahmoud<sup>b</sup>, Cyril Viarouge<sup>c</sup>, Corinne Sailleau<sup>c</sup>, Stephan  
4 Zientara<sup>c</sup>, Emmanuel Breard<sup>c\*</sup>

5 <sup>a</sup>Department of Cell Biology, Genetic Engineering and Biotechnology Division, National Research  
6 Centre, 12622 Dokki, Giza, Egypt

7 <sup>b</sup>Department of Parasitology and Animal Diseases, Veterinary Research Division, National Research  
8 Centre, 12622 Dokki, Giza, Egypt

9 <sup>c</sup> Université Paris Est, ANSES, ENVA, INRA, UMR 1161 VIROLOGIE, Laboratory for Animal Health,  
10 Maisons-Alfort, France.

11  
12 \*Correspondence: Emmanuel.Breard@anses.fr

13  
14 **Short title:** BT and EHD viruses in Egypt

15  
16 **Abstract**

17 BTV and EHDV are closely-related orbiviruses that are transmitted between domestic and  
18 wild ruminants via the bites of hematophagous midges. Previous studies have reported seropositivity  
19 against BTV antibodies in sheep and goats in two Egyptian governorates (Beni Suef and Menoufia).  
20 However, no recent data are available on the BTV serotype(s) circulating in Egypt and the likely  
21 presence of EHDV has never been explored. This study investigated the presence of BTV and EHDV  
22 among cattle which had been found BTV-seropositive by ELISA method. These cattle living in  
23 proximity to sheep and goats previously found BTV-seropositive. These cattle displayed no clinical  
24 signs of BT but reproductive problems had been reported in herds. A total of 227 cattle blood  
25 samples were therefore collected in 2016 and 2017. Ninety-four of the 227 animals tested by a BTV  
26 ELISA were positive for BTV antibodies (41.4%). Of these 94 ELISA-positive cattle, only 83 EDTA-blood  
27 samples were available and therefore tested for BTV and EHDV genome detection by RT-PCR and  
28 sequencing.

29 Of the cattle sampled in 2016, results revealed that two were RT-PCR-positive for BTV and  
30 seven for EHDV. Sequencing showed the presence of EHDV-1 and BTV-3 genome sequences. EHDV-1  
31 S2 shared 99.5% homology with an EHDV-1 S2 from a strain isolated in 2016 in Israel. BTV-3 S2 and  
32 S8 sequences shared more than 99.8% nucleotide similarity with the BTV-3 Zarzis S2 and S8  
33 sequences (Tunisian BTV, also detected in 2016). Of the 66 blood samples tested following their  
34 collection in 2017, they were all EHDV-negative by RT-qPCR while five were BTV- positive by RT-qPCR.  
35 However, attempts to identify the BTV serotype of these five samples were unsuccessful. Only part of  
36 BTV S8 was sequenced and it showed 79% nucleotide similarity with S8 of atypical BTV serotypes  
37 (particularly with BTV-26 and another BTV serotype strain isolated from a sheep pox vaccine).

38 Overall, these findings demonstrate that both BTV and EHDV were circulating in Egypt in 2016 and  
39 2017.

40

41 **Abbreviations:** BTV: Bluetongue virus; EHDV: Epizootic hemorrhagic disease virus; EHDV-1: EHDV  
42 serotype 1; BTV-3 or -26: BTV serotype 3 or 26; S: Segment; q: Real-time

43

44 **Keywords:** BTV, EHDV, Egypt, ELISA, genome detection

45

46 **1. Introduction**

47 Bluetongue (BT) and Epizootic hemorrhagic disease (EHD) are vector-borne viral diseases  
48 affecting domestic and wild ruminants, notifiable under OIE rules. BT and EHD viruses are species of  
49 the genus *Orbivirus*, with structural, antigenic and molecular similarities. Their genomes are  
50 composed of ten segments (S1 to S10) of double-stranded RNA which encode seven structural  
51 proteins (VP) and five nonstructural proteins (NS) (Ratinier et al., 2011). VP2 forms the outer capsid,  
52 and is therefore a target of neutralizing antibodies, as well as determining the virus serotype.

53 Both viruses induce variable clinical signs that mainly depend on the strain and the affected  
54 ruminant species (or breed). Both diseases have been reported to involve seasonal circulation and  
55 transmission by hematophagous midges (*Culicoides*) (Anthony et al., 2009; Maclachlan et al., 2009).  
56 Twenty-four traditional BTV serotypes in addition to novel and atypical serotypes have been  
57 identified to date (Bumbarov et al., 2016; Chaignat et al., 2009; Lorusso et al., 2018; Maan et al.,  
58 2011; Marcacci et al., 2018; Savini et al., 2017; Sun et al., 2016; Zientara et al., 2014). Since 2009,  
59 there has been a consensus recognizing seven EHDV serotypes (1, 2, and 4 to 8) (Anthony et al.,  
60 2009).

61 The global distribution of BT and EHD has recently expanded, possibly due to a variety of factors  
62 including increased global commerce and climate change (Guis et al., 2012; Maclachlan et al., 2009;  
63 Wilson and Mellor, 2009). In the last 20 years, various BTV serotypes have been reported in Europe  
64 and other countries bordering the Mediterranean basin, whereas EHDV has sporadically been  
65 detected in countries in the south or east of the Mediterranean basin (the Maghreb, Israel, Turkey,  
66 and Jordan) (Ben Dhaou et al., 2016; Temizel et al., 2009).

67 From 1998 to 2005, at least seven BTV incursions (involving five BTV serotypes (serotypes 1, 2, 4,  
68 9 and 16)) spread across the Mediterranean basin (the Maghreb, Mediterranean islands and  
69 Southern Europe), inducing clinical signs in sheep and goats. These BTV strains entered the  
70 Mediterranean Basin and Europe through two main corridors: eastern via Turkey and Greece and

71 western via North Africa (Lorusso et al., 2014). In this last case, BTV first spread through North Africa,  
72 then across multiple Mediterranean islands and finally, across southern European countries (Spain,  
73 Italy and/or France).

74 In 2006, a BTV-8 strain emerged in Northern Europe and rapidly infected naive ruminants across  
75 northern and eastern European countries. It was not only virulent in sheep but also, unexpectedly, in  
76 cattle and its origin is still unknown. Experimental and field studies conducted on a large number of  
77 abortions reported transplacental transmission of BTV-8 in cattle (Zanella et al., 2012). After  
78 compulsory nationwide vaccination campaigns, Europe became free of BTV-8 in 2012. However, the  
79 same BTV-8 strain re-emerged in 2015 in France (Breard et al., 2016), has since spread and is now (in  
80 2019) present in mainland France, Switzerland and eastern Germany. A BTV-4 strain from Eastern  
81 Europe (detected in Greece in 2014) moved westwards (through Balkan countries) and has been  
82 found in Italy and mainland France in 2019 (Sailleau et al., 2018).

83 In 2018, an exotic serotype (BTV-3) was detected in sheep in Sardinia. This strain was shown  
84 to be almost identical (>99% nucleotide identity) across all segments to the BTV-3 strain isolated in  
85 2016 in Tunisia (Cappai et al., 2019). This new BTV-serotype incursion in the Mediterranean Basin,  
86 spreading northwards, bears resemblance with the distinctive corridor where BTV-1, -2 and -4 strains  
87 had previously been observed spreading from Africa into Europe (Lorusso et al., 2013). Indeed,  
88 serotype 3 constitutes a possible future threat for Southern Europe.

89 Common to each *Orbivirus* is the variability of clinical outcomes after infection. In many cases,  
90 BTV and EHDV induce mild or unapparent clinical infections, whereas in others they induce fever,  
91 depression, respiratory distress and anorexia (MacLachlan et al., 2008; Maclachlan et al., 2009; Savini  
92 et al., 2017). In the case of BTV, sheep are the most sensitive species. Although goats and cattle are  
93 fully susceptible to BTV infection, they do not generally show signs of disease; it remains mild or  
94 subclinical. Cattle show longer periods of BTV viremia than sheep and are considered reservoirs of  
95 the virus. Experimental and field studies have reported clinical signs of BT in cattle and goats,

96 particularly when outbreaks have occurred in non-endemic areas (MacLachlan, 2004). Historically,  
97 EHDV has been associated with disease in wild cervids (Jessup, 1985; Odiawa et al., 1985). Severe  
98 clinical signs induced by EHDV have also been observed in infected cattle (Maclachlan et al., 2015;  
99 Omori et al., 1969). EHDV-6 or -7 has sporadically spread in countries surrounding the Mediterranean  
100 Basin and has induced clinical forms in cattle (Ben Dhaou et al., 2016; Temizel et al., 2009; Yadin et  
101 al., 2008). More precisely, in 2006, outbreaks of EHDV-6 were reported in cattle from Turkey,  
102 Morocco, Algeria, Tunisia and Jordan when an EHDV-7 strain was involved in outbreaks in Israel. The  
103 more recent outbreaks of EHD in the Mediterranean Basin were recognized clinically in 2015 In Israel  
104 involving an EHDV-6 strain (Golender et al., 2017). The introduction origins of these EHDV-6 and 7  
105 strains detected in Mediterranean countries since 2006 are still unknown, however, analyses of their  
106 genome segments indicate a common “African/Arabian Peninsula and Indian Ocean Asia” origin (Ben  
107 Dhaou et al., 2016; Golender et al., 2017).

108         Studies concerning the presence of orbiviruses have been conducted in various countries  
109 neighboring Egypt and have confirmed the endemic nature of BT and EHD in these areas (Mejri et al.,  
110 2018; Sghaier et al., 2017). In Egypt, several serological studies reported BTV seroprevalence  
111 (Mahmoud and Khafagi, 2014; Mahmoud et al., 2017). In 1977, a BTV-4 strain from Egypt was  
112 isolated by the Pirbright Institute (ReOId). In 1987, BTV serotypes 1, 4, 10, 12 and 16 were identified  
113 through seroneutralization assays in retrospective studies from Egyptian sera (Ismail et al., 1987).  
114 Recent investigations carried out on sheep and goats have revealed the presence of antibodies  
115 against BTV in these domestic species in two Egyptian governorates (Beni Suef and Menoufia)  
116 (Mahmoud et al., 2017). Egyptian veterinary authorities wanted to determine whether BTV could  
117 have been involved in the reproductive problems reported in 2016 and 2017 in several herds located  
118 in these two Egyptian governorates. This study reports the results of the molecular detection of BTV  
119 and EHDV that was carried out on cattle samples collected in this context.

120

121 **2. Material and methods**

122 **2.1. Animals and samples collected**

123 A total of 227 cattle blood samples were collected from two herds in Beni Suef (72 animals  
124 sampled in 2016) and three herds in Menoufia (155 animals sampled in 2017). The animals were  
125 apparently healthy, but reproductive problems had been reported in these herds. Serum and EDTA-  
126 blood samples were stored in the National Research Centre (NRC) (Egypt) at -20°C until use.

127

128 **2.2. ELISA**

129 The ID Screen Bluetongue Competition ELISA kit (IDVet, Grabels, France) was used as per the  
130 manufacturer's instructions to initially detect BTV VP7 antibodies in all 227 serum samples. Eighty-  
131 three EDTA-blood samples from ELISA-positive cattle were then used for the molecular diagnosis of  
132 BTV and EHDV.

133

134 **2.3. RNA extraction**

135 A first batch of 17 RNA extracts came from 17 EDTA-blood samples collected in 2016 in Beni Suef.  
136 This RNA was purified in Egypt by the National Research Centre (NRC) using the TRIzol method. Two  
137 hundred and fifty µl of EDTA-blood was extracted using TRIzol LS Reagent as per the manufacturer's  
138 instructions. Total RNA was eluted into 30 µl of RNase-free water. The extraction products were then  
139 sent to ANSES's Laboratory for Animal Health (Maisons-Alfort, France) for RT-PCR assays and  
140 sequencing.

141 The second batch consisted of 66 EDTA-blood samples (from ELISA-tested cattle found positive  
142 for BTV) sent to ANSES's Laboratory for Animal Health in order for RNA extraction to be carried out  
143 using the MagVet Universal Isolation kit (Thermo Fisher Scientific, Lissieu, France). Total RNA was  
144 then extracted from 100 µl of EDTA-blood using an automated method (KingFisher Flex automated

145 extraction platform (ThermoFisher Scientific) and the MagVet Universal nucleic acid extraction kit  
146 (Thermo Fisher)). Total RNA was eluted into 80 µl of RNase-free water.

147

#### 148 **2.4. RT-PCRs**

149 Five µl of denatured RNA (heated at 95°C for 3 minutes) was then tested using each RT-PCR  
150 method (real-time RT-PCR (RT-qPCR) or conventional RT-PCR). Commercial RT-qPCR kits were used  
151 for pan-BTV detection (Adiavet BTV kit (Bio-X Diagnostics, Saint Briec, France)) and for detecting  
152 BTV-1, -2, -4, -8, -9 or -16 (serotypes currently found in Europe) (VetMAX European BTV Typing Kit),  
153 as per the manufacturer's instructions. An in-house BTV-3 RT-qPCR (Lorusso et al., 2018) was also  
154 used.

155 In-house RT-qPCRs were used to specifically detect the EHDV genome; these RT-qPCRs were EHDV-  
156 group or -serotype specific (Viarouge et al., 2015).

157 For each of the in-house RT-qPCRs targeting EHDV or BTV segments, different primers and  
158 probe sets were designed in order to amplify part of the targeted gene using the AgPath One-Step  
159 RT-qPCR Kit (Life Technologies). Amplifications were carried out using the same cycling parameters  
160 for all RT-qPCR assays: 45°C for 10 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s then  
161 60°C for 1 min. These same cycling parameters were also applied when using commercial RT-qPCR  
162 kits: in-house RT-qPCR methods have been developed to be used together, in a single amplification  
163 run that could incorporate various commercial RT-qPCR mixes. Conventional RT-PCRs for BTV S2, S8,  
164 S9 and S10 sequence amplifications (Breard et al., 2007; Sailleau et al., 2012; Viarouge et al., 2014)  
165 were also performed using the Qiagen OneStep RT-PCR kit (Qiagen, France). The amplification  
166 products were directly sequenced, in both directions, according to the Sanger method by Eurofins.

167

#### 168 **2.5. Phylogenetic analyses**



169 Sequences were assembled and analyzed using EditSeq, SeqMan and Megalign Software (DNA  
170 Star Inc.) and aligned using ClustalW (DNASTar programs, Lasergene). The EditSeq program was used  
171 to split the sequence into individual files, SeqMan was used to assemble the reads of each sequence  
172 into contigs, and the Megalign program was then used to calculate sequence relationships, compare  
173 phylogenetic associations among BTV strains and construct phylogenetic trees based on the  
174 nucleotide sequences. The Megalign program uses the Neighbor-Joining method based on a matrix of  
175 “distances” between all sequences. The phylogenetic trees generated by Megalign are rooted trees,  
176 regardless of the method used for alignment.

177

## 178 **2.6. Isolation assays**

179 Isolation assays were carried out (at NRC and ANSES) on embryonated eggs (Clavijo et al., 2000)  
180 and KC cells (*Culicoides sonorensis* cell line)(Wechsler et al., 1989) using blood samples found positive  
181 for BTV or EHDV by RT-qPCR. The blood samples were inoculated onto KC cells. Briefly, a confluent  
182 monolayer of KC cells was inoculated with EDTA-blood samples diluted  $10^{-1}$  in sterile PBS. The  
183 inoculum was removed 24 hours after inoculation and the cells were incubated with an appropriate  
184 cell culture medium (Schneider's Drosophila Media (ThermoFisher Scientific) and 10% of foetal calf  
185 serum). After 7 days of incubation at 28°C, KC cell culture supernatants were tested for the presence  
186 of BTV or EHDV RNA by RT-qPCR.

187 In parallel, groups of three embryonated chicken eggs were each inoculated intravenously with  
188 0.1–0.2 ml of a  $10^{-1}$  dilution of lysed blood samples. The eggs were incubated for 7 days at 35°C and  
189 examined daily using a cold candling lamp. The embryos that died within 24 h were discarded. After 1  
190 week of incubation, the embryos were weighed and homogenized in Minimum Essential Medium  
191 (weight/volume: 1 / 10). Total RNA was then extracted from 100 µl of embryo lysate and tested by  
192 EHDV or BTV RT-qPCR.

193

194 **3. Results and discussion**

195 Serological analysis by ELISA revealed that 41.4% (94/227) of blood samples tested using the VP7  
196 competitive ELISA were positive for BTV antibodies (Table 1). In each governorate, the percentage of  
197 cattle found positive for BTV by ELISA was similar (Table 1).

198 The BTV genome was looked for only in these ELISA-positive cattle. This limit was imposed  
199 due to findings from previous studies: when serum and EDTA-blood from BTV-infected animals (in  
200 the field) were tested, the animals that tested positive for BTV by RT-qPCR were mainly also found  
201 positive for BTV by the ELISA method (Breard et al., 2005; Verdezoto et al., 2018; Viarouge et al.,  
202 2014). An ELISA (commercial kit) is easy to perform in any laboratory and in our studies enables non-  
203 infected herds to be set aside in order not to undergo molecular analyses. Furthermore, when  
204 samples come from potentially endemic areas of BTV (Ecuador, Galapagos Islands and French Guiana  
205 in South America, Reunion Island in the Indian Ocean, and Tunisia in Africa), we systematically look  
206 for the EHDV genome or its antibodies (Ben Dhaou et al., 2016; Breard et al., 2004; Breard et al.,  
207 2005; Sailleau et al., 2012; Verdezoto et al., 2018; Vinueza et al., 2019). In all these studies, EHDV was  
208 always present in areas where BTV was also found. It is also common to find animals co-infected by  
209 both viruses.

210 Eighty-three EDTA-blood samples from these 94 ELISA-positive cattle were available and tested  
211 for BTV and EHDV genome detection by RT-qPCR. Of the first 17 RNA extracts sampled in 2016 and  
212 sent to ANSES, two head of cattle (C19 and C44) were positive by BTV group-specific RT-qPCR (Table  
213 2). Both of these samples were also found to be positive by RT-qPCR specific to BTV-3 and negative  
214 by the RT-qPCR that amplified BTV-1, -2, -4, -8, -9, and -16. S2 (VP2) and S8 (NS2) sequences (754 bp  
215 (GenBank Accession no. MH706764) and 942 bp (GenBank Accession no. MH706766) respectively)  
216 were obtained by conventional RT-PCR from C44 RNA, showing 99.86% and 99.89% homology with  
217 the Tunisian BTV-3 strain (Zarzis) isolated in 2016 (Sghaier et al., 2017). A phylogenetic tree (Figure 1)  
218 illustrates the homology between Egyptian BTV-3 S2 and the S2 from BTV-3 strains isolated across

219 the world. These results demonstrate that the BTV-3 variant detected in Tunisia in 2016 was also  
220 present in Egypt that same year and supports the hypothesis that BTV-3 Zarzis had moved westwards  
221 after originating from North-East Africa.

222 A particular surprise was to detect a specific EHDV genome in seven cattle blood samples  
223 collected in 2016. EHDV-1-specific RT-qPCR (Viarouge et al., 2015) allowed the EHDV serotype to be  
224 determined for six of the seven pan-EHDV-positive cattle (Table 2). Only the sample with a Ct of 34.6  
225 was not positive by EHDV-1 RT-qPCR, no doubt due to its low viral load. The tree is based on a single  
226 sequence sample (longer sequences were obtained with RNA from C17). The serotype was confirmed  
227 using conventional RT-PCR in three out of the seven EHDV-positive cattle and sequencing results  
228 demonstrated that the amplification products (1839 bp, GenBank Accession no. MH706767) shared  
229 99.5% homology with S2 of an EHDV-1 strain isolated in 2016 in Israel (GenBank Accession no.  
230 MG808409), 95.86% homology with an EHDV-1 S2 isolated in Nigeria in 1967 and less than 87% with  
231 other known EHDV-1 S2 sequences available in GenBank (Figure 2). One S2 sequence was obtained  
232 from C20 and C21 (about 800 bp) with 100% homology with sequence S2 from C17. To our  
233 knowledge, this study is the first report of EHDV-1 in North Africa. EHDV-6 and 7 have previously  
234 been found in the Maghreb, Turkey and Israel (Ben Dhaou et al., 2016; Temizel et al., 2009; Yadin et  
235 al., 2008) whereas EHDV has never yet been reported on any Mediterranean islands or in Europe.

236 Out of the 66 blood samples collected in 2017 in Menoufia and extracted in ANSES's  
237 laboratory, five were found positive for BTV by RT-qPCR and all were negative for EHDV by RT-qPCR  
238 (Tables 1 and 2). No amplification product was obtained when either classical or serotype-specific RT-  
239 qPCR targeted BTV S2. In this study, the authors tried to determine the BTV serotype(s) by RT-qPCR  
240 or conventional RT-PCR. Finding no success for some samples, we subsequently tried to amplify and  
241 sequence BTV segments other than S2 (easier to amplify because they have more conserved  
242 nucleotide sequences than S2) (Breard et al., 2005; Sailleau et al., 2012). The objective was then to  
243 confirm, using a BTV genome sequence, the positive results obtained for the group by pan-BTV RT-

244 qPCR (targeting S10). Indeed, we tried to amplify S8, S9, and S10 , but only segment 8 amplification  
245 worked for one out of the five BTV-positive animals sampled in 2017 (914 bp, GenBank Accession no.  
246 MH706765). The S8 sequence showed 79.86% homology with S8 of a BTV strain isolated from a  
247 sheep pox vaccine (Bumbarov et al., 2016) and 78.99% with BTV-26 (Maan et al., 2011) (data not  
248 shown). These five RT-qPCR-positive blood samples obtained in 2017 were also negative with BTV-3  
249 RT-qPCR. Considered as a whole, the BTV RT-PCR results suggest that other BTV serotypes were able  
250 to circulate at least in the Menoufia governorate in 2017, and that one of these BTV serotypes was  
251 atypical, like many other similar BTV serotypes recently discovered in the Mediterranean Basin and  
252 Europe (Chaignat et al., 2009; Lorusso et al., 2018; Schulz et al., 2016; Sghaier et al., 2017).

253           Unfortunately, no virus was isolated from EHDV- or BTV-positive blood samples, most likely  
254 due to their pre-analysis storage at -20°C or the fact that animals were sampled after the viremia  
255 period, when the virus had been neutralized but viral RNA was still present in the blood and  
256 detectable by RT-PCR. Isolating EHDV or BTV strains in most cases allowed us to obtain full EHDV or  
257 BTV genomes by the NGS method (Breard et al., 2016; Schulz et al., 2016; Verdezoto et al., 2018).  
258 Obtaining the full genome for every strain would have allowed us to verify that the Egyptian BTV-3,  
259 which has the same S2 and 8 as the Tunisian Zarzis strain, was not a reassortant virus for the other  
260 eight RNA segments.

261

#### 262 **4. Conclusions**

263           This study showed that BTV-3 (Zarzis strain) and EHDV-1 were present in Egypt in 2016 in the  
264 same period during which BTV-3 was detected in Tunisia and EHDV-1 in Israel. These data confirm  
265 that Egypt constitutes an area of particular interest for investigating BTV and EHDV epidemiology in  
266 the Mediterranean Basin. Additional studies are necessary in order to estimate BTV and EHDV  
267 seroprevalence in this part of Africa, and to isolate BTV and EHDV strains so as to characterize the full  
268 genome of these orbiviruses present in the Mediterranean basin. Further collaboration must be

269 established between European and Mediterranean countries in order to control the spread of BTV  
270 more easily and effectively.

271

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274

275 **Conflict of Interest Statement:** Declarations of interest: none.

276

277 Table 1

278

year of sampling	governorate	BTV ELISA				PCR		
		number of cattle tested	positive	negative	% of positive cattle	number of ELISA-positive cattle tested	BTV-positive	EHDV-positive
2016	Beni Suef	72	28	44	38.9	17	2	4
2017	Menoufia	155	66	89	42.6	66	5	0
	Total	227	94	133	41.4	83	7	4

279

280

281

282 Table 2

283

Location	Date of sampling	Animal n°	Ct value with pan-BTV PCR	Ct value with pan-EHDV PCR	Serotype
Beni Suef	13/10/2016	C44	19.1	-	BTV-3 <sup>a b</sup>
	6/11/2016	C13	-	30	EHDV-1 <sup>a</sup>
		C17	-	26.7	EHDV-1 <sup>a b</sup>
		C19	31.6	-	BTV-3 <sup>a</sup>
		C20	-	26.8	EHDV-1 <sup>a b</sup>
		C21	-	26	EHDV-1 <sup>a b</sup>
		C7	-	34.6	undetermined
		C1	-	29.7	EHDV-1 <sup>a</sup>
		C9	-	27	EHDV-1 <sup>a b</sup>
Menoufia	27/07/2017	C60	31.7	-	undetermined
		C61	36.9	-	undetermined
		C69	29.4	-	undetermined <sup>c</sup>
		C86	31.2	-	undetermined
		C87	30.9	-	undetermined

284 <sup>a</sup>: determined with RT-qPCR.

285 <sup>b</sup>: determined with sequencing.

286 <sup>c</sup>: only NS2 sequence obtained.

287

288 **Captions**

289 Table 1: ELISA and PCR results from 277 Egyptian cattle sampled in 2016 and 2017 in two Egyptian  
290 governorates.

291

292 Table 2: RT-PCR and sequencing results of the 14 head of cattle found positive for BTV or EHDV by  
293 RT-qPCR sampled in 2016 and 2017 in Beni Suef and Menoufia governorates.

294

295 Figure 1: Phylogenetic tree of full-length segment 2, showing the relationships between EHDV-1 2016  
296 Egypt (MH706767) segment 2 (bold letters) and homologous EHDV serotypes (Genbank). The  
297 phylogenetic tree of nucleotide sequences was constructed using MegAlign Clustal W method  
298 (DNASTAR software, Lasergene 8).

299

300 Figure 2: Phylogenetic tree of full-length segment 2, showing the relationships between BTV-3 Egypt  
301 (MH706764) segment 2 (bold letters) and homologous BTV-3 serotypes (Genbank). The phylogenetic  
302 tree of nucleotide sequences was constructed using MegAlign Clustal W method (DNASTAR software,  
303 Lasergene 8).

304

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Figure 1

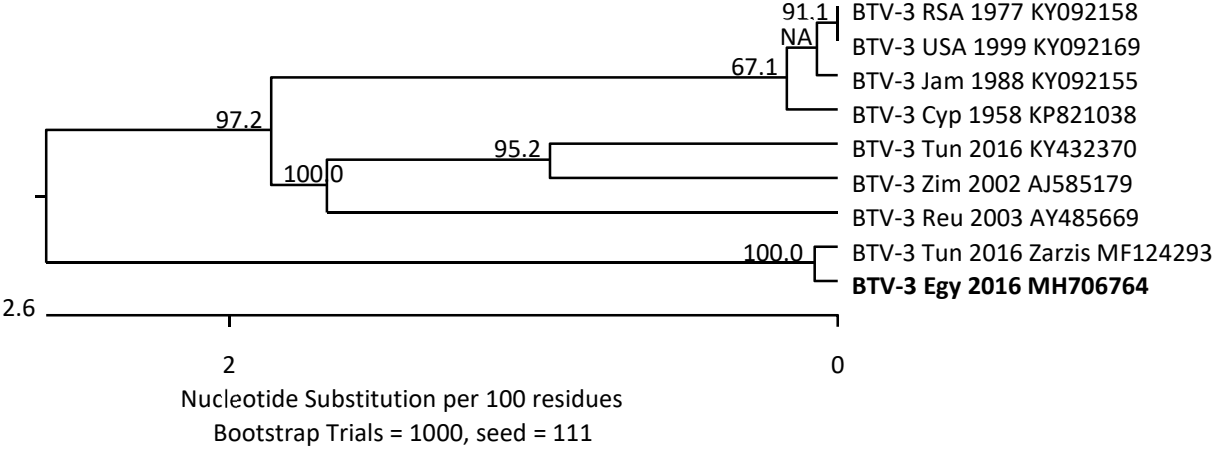


Figure 2

