

1 **Title:** First Detection of Epizootic Haemorrhagic Disease virus in the European Union, Italy-2022.

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15 **Running Title:** EHDV in the EU

16 **Biography of the first author:** Alessio Lorusso, DVM-PhD is active in surveillance and characterization of
17 viruses of public health interest.

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29 **Abstract**

30 We describe the first detection in the European Union of the epizootic haemorrhagic disease virus (EHDV).
31 EHDV-8 has been detected in cattle farms in Sardinia and Sicily. The virus has a direct Northern African
32 origin as its genome is identical (>99.9% nucleotide sequence identity) to EHDV-8 strains detected in Tunisia
33 in 2021/2022.

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62 Introduction

63 The epizootic haemorrhagic disease (EHD) is a WOAHL-listed disease of wild and domestic ruminants caused
64 by EHD virus (EHDV). EHDV infection in deer, particularly among white-tailed deer (*Odocoileus virginianus*)
65 in North America, can cause high levels of mortality. EHDV is related to the bluetongue virus (BTV),
66 etiological agent of the bluetongue disease of ruminants (BT). Both viruses belong to the genus *Orbivirus*
67 (family *Sedoreoviridae*) and circulate in multiple serotypes (1,2). Their viral genome comprises 10 linear
68 segments (S1-S10) of double-strand RNA and the structural outer capsid protein (coded by S2) determines
69 serotype specificity. Both viruses cause similar clinical signs in cattle and are transmitted by several species
70 of biting midges of the genus *Culicoides*. BT primarily affects sheep and has been described multiple times
71 in the last twenty-four years in the European Union (EU) causing devastating outbreaks in ruminants with
72 repercussions on animal trade (3). Most European BT outbreaks had a direct Northern African origin
73 because of wind-driven dissemination of BTV-infected midges from these areas (1, 4-7).

74 Here, we describe the detection of EHDV-8 in cattle in Sardinia and Sicily, Italy. This represents the first
75 evidence of EHD in the European Union (EU). This report follows our previous studies on EHDV-8 in Tunisian
76 cattle in 2021 (8).

77 Materials and Methods

78 On October 28 2022, clinical signs suggestive of BT infection (inappetence, cyanosis and edema of the
79 tongue, conjunctivitis, and fever) were reported by the Local Veterinary Services (LVS) in one cattle from a
80 farm (F) 1 (Fig. 1A) located in the municipality of Arbus (Fig. 1B). On November 3, the animal succumbed. At
81 necropsy, spleen was collected by the LVS along with blood samples from three additional symptomatic
82 cattle. On November 4 2022, in F2 and F3 (Arbus and Guspini, respectively Fig. 1B) three cattle showed the
83 same symptomatology (Fig. 1C). On October 25 2022, BT-like clinical signs were also evidenced in three
84 cattle belonging to F4 located nearby Trapani (Sicily, Fig. 1D). Samples were collected from the described
85 animals and tested for the presence of EHDV RNA (VetMAX™ EHDV Kit rRT-PCREHDV; Thermo Scientific™
86 Waltham, MA, USA). A real time RT-PCR specific for the S2 of EHDV-8 TUN 2021 (rRT-PCR_{EHDV-8}) was
87 established (Portanti et al., manuscript in preparation) as the available test designed on the S2 of the EHDV-
88 8 reference serotype (Australia, 1982) was not able to detect EHDV-8 TUN 2021 (8). Overall, nucleotide
89 sequences of forward and reverse primers and probes are EHDV_Ser8varNEW_fwd
90 AGAGATGAAGATCGCGAGGA (975-994); EHDV_Ser8varNEW_rev GAATCACACGCGCTACTAA (1135-1159)
91 and EHDV_Ser8varNEW_Probe FAM-ACGGATGAGATACGGAACATACGGGG-TAMRA (1066-1091),
92 respectively. Whole genome sequencing-WGS (9) was performed on selected samples to get information
93 upon the genome constellation of the occurring strain.

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95 **Results**

96 All sampled animals from Sardinia and Sicily were positive for EHDV (Ct range 23-28). Genotyping by a
97 specific S2 EHDV-8 TUN2021 real-time RT-PCR confirmed the presence of a EHDV-8 TUN2021-like strain.
98 The evidence of EHDV was notified to the Italian Ministry of Health which in turn notified the WOA and
99 the European Commission. This implied the ban of animal (all ruminants) movement from the two islands
100 and the establishment of a 150 km radius restriction zone around the outbreaks. One EHDV-8 positive
101 blood sample from Sardinia was selected for WGS. Genome analysis confirmed that the Sardinian EHDV-8
102 strain (EHDV-8 SAR2022 NCBI Submission ID: 2646388) shares the same genome constellation and high
103 nucleotide sequence relatedness (>99.9%) with multiple EHDV-8 TUN 2021-like strains sequenced so far.
104 WGS for Sicilian strains is currently ongoing.

105 **Discussion**

106 A novel *Orbivirus* incursion to the EU sustained by EHDV-8 is reported. This virus has a direct Northern
107 African origin. This event was reasonably predictable as for the current widespread distribution of the same
108 virus in Tunisia and likely in neighboring countries (8), and in consideration of the previous incursions of
109 multiple BTV strains to Southern Europe. At the time this report has been prepared, on November 18 2022,
110 EHD has been notified also in Andalusia (Cadiz and Sevilla)-Spain. At this point, it is hard to predict the
111 future scenarios for the EU cattle production system and the impact of the possible spread of EHDV. EHD
112 will probably pose new challenges that the EU veterinary authorities will be forced to face. The lessons
113 learned with BT should be a reference for choosing proper control and prevention strategies for EHD.
114 Overall, these events further emphasize the importance for European countries in general, and for Italy due
115 to its geographical location, of having in place robust collaborations with Northern African authorities on
116 public and animal health. The prompt detection of EHDV-8 in Sardinia and Sicily is, indeed, the last example
117 of the benefits that could derive from such relationships. This was crucial, as it facilitated the development
118 of a specific and accurate molecular test for the detection of EHDV-8 as knowledge upon the genome
119 constellation and the genomic relatedness of EHDV-8 with extant EHDV serotypes was already achieved.
120 Undoubtedly, vaccine development needs to be boosted as vaccination is the only strategy to prevent
121 direct and indirect economic losses and significantly reduce virus circulation.

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153 **Conflict of Interest**

154 The authors disclose any conflicts of interest.

155 **Ethical approval**

156 No ethical approval was required as biological samples from animals were collected during outbreak
157 investigations.

158

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164 “*Detection of mosquito and Culicoides-borne viruses in Sardinia by innovative NGS-based techniques and*
165 *evaluation of Bluetongue virus evolution*”.

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167 The mention of commercial products in this article is simply for the purpose of providing specific
168 information and does not imply a recommendation or approval by IZS-Te.

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170 **Figure legend**

171 **Fig 1.** Geographical locations (Google Earth) of the four farms involved in the early outbreaks and clinical
172 signs observed in cattle (cyanosis and edema of the tongue).

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Sardegna

Cagliari



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1C

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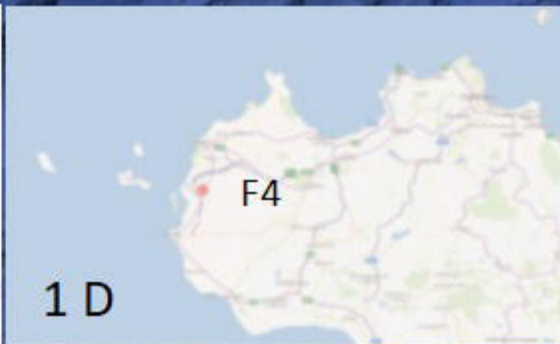


F2

F3

F1

1 B



F4

1 D



F4

Trapani

Palermo

Sicilia

Catania

Siracusa

Messina

Tunisi تونس