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Jacques Fourgeaud, Audrey Mirand, Justine Demortier, Laure Kamus, Louis Collet, Sophie Olivier, Cécile Henquell, Christelle Vauloup-Fellous

► **To cite this version:**

Jacques Fourgeaud, Audrey Mirand, Justine Demortier, Laure Kamus, Louis Collet, et al.. Enterovirus meningitis in Mayotte French Comoros Island, March-June 2019. *Journal of Clinical Virology*, 2022, 150-151, pp.105154. 10.1016/j.jcv.2022.105154 . hal-03850956

**HAL Id: hal-03850956**

**<https://uca.hal.science/hal-03850956v1>**

Submitted on 22 Jul 2024

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1 **Manuscript Number** (JCV-D-21-01120)

2

3 **Title: Enterovirus meningitis in Mayotte French Comoros Island, March-June, 2019**

4 **Authors:**

5 Jacques Fourgeaud<sup>1</sup>, Audrey Mirand<sup>2</sup>, Justine Demortier<sup>3</sup>, Laure Kamus<sup>3</sup>, Louis Collet<sup>3</sup>, Sophie  
6 Olivier<sup>3</sup>, Cécile Henquell<sup>2</sup>, Christelle Vauloup-Fellous<sup>4</sup>

7 **Affiliations**

8 1 AP-HP, Necker Enfants Malades Hospital, Virology department, EA 7328 PACT, Institut  
9 Imagine, Université de Paris, F-75015 Paris, France

10 2 CHU Clermont-Ferrand, 3IHP, Virology Department, National Reference Laboratory for  
11 Enterovirus and Parechovirus; Clermont Auvergne University, CNRS, LMGE, Clermont-  
12 Ferrand, France

13 3 Laboratoire de biologie, Centre Hospitalier de Mayotte, Mamoudzou, Mayotte, France

14 4 AP-HP, Université Paris Saclay, Hôpital Paul Brousse; Virology department, INSERM U  
15 1193; Univ Paris Saclay, Villejuif, France

16

17 **Corresponding author** : Jacques Fourgeaud, Laboratoire de Microbiologie, Hôpital

18 Universitaire Necker Enfants Malades, 149 rue de Sèvres, 75015 Paris, France; email:

19 jacques.fourgeaud@aphp.fr; tel : +336 72 08 86 05; fax: +331 44 49 49 60

20 **Abstract—244 words**

21 **Background:** Enteroviruses (EV) are the most frequent cause of acute meningitis worldwide,  
22 and regularly responsible for outbreaks. Human parechoviruses (PeV) are associated with sepsis  
23 and meningitis in young infants. In Mayotte, a French department located in the Comoros  
24 archipelago, EVs and PeVs are not part of the routine screening of cerebrospinal fluids (CSFs) of  
25 patients with meningitis. Consequently, no data is available on EV or PeV epidemiology.

26 **Aim:** Assess the need for EV and PeV diagnosis in Mayotte.

27 **Methods:** CSFs collected between March and June 2019 from patients addressed to Mayotte  
28 Hospital were retrospectively screened for EV and PeV by PCR. If positive for EV, genotyping  
29 was attempted.

30 **Results:** EV and PeV RT-PCR were performed on 122/263 (46%) CSFs (45 adults, 77 children).  
31 EV meningitis was diagnosed in 16/77 children (21%) with a median age of 32 days (8-62). One  
32 30-days-aged infant presented with a PeV infection. Fever was reported in 94% cases (16/17),  
33 followed by gastrointestinal disorders in 29% cases (5/17). EV genotyping achieved  
34 identification for 10/16 (63%) EV-positive samples. Four different EV types were identified:  
35 Echovirus 16 (E-16, n=6), EV-B100 (n=2), and E-14 and E-18 (n=1, each).

36 **Conclusion:** EV/PeV prevalence of 14% highlights the importance of implementing this  
37 diagnosis which can impact duration of hospitalization and administration of antibiotics thus  
38 reducing risk of antimicrobial resistance. Surveillance of circulating EV types is needed to  
39 understand the range of enteroviruses detected in meningitis cases in places that have been  
40 underrepresented in enterovirus surveillance studies.

41

42 **Keywords—6 words :**

43 Mayotte; molecular epidemiology; meningitis; Echovirus; Enterovirus; Parechovirus

44

45 **Text—2475 words**

46 **Introduction**

47 Human enteroviruses (EVs) and parechoviruses (PeVs) are RNA viruses of the  
48 *Picornaviridae* family (1). These viruses, like most positive-sense single-stranded RNA viruses,  
49 are characterized by high genetic diversity resulting from the continuous generation of mutations  
50 by the viral RNA polymerase, and genetic recombination events (2). More than 100 EV types  
51 types in species A to D are known to infect humans (3). PeVs belong to one of the two species of  
52 *parechovirus* genus among which 16 different types (PeV-1 to PeV-16) have been identified (4).

53 Enteroviruses are distributed worldwide, and have a seasonal circulation pattern in  
54 temperate countries, causing peaks of infections in early and late summer (5). In tropical  
55 countries, circulation of EVs, although less studied, is year-round with a higher frequency during  
56 the rainy season (5). Usually, EVs and PeVs infections are asymptomatic or associated with mild  
57 symptoms but are also the main agents involved in aseptic meningitis, especially in children (6).  
58 If most EV meningitis are benign, some patients may present with central nervous system  
59 complications depending on age, or on the EV type involved (7). Additionally, neonatal EV  
60 infections can be associated with severe, potentially fatal sepsis-like conditions. Circulating

61 genotypes differ over time and location (8,9)(6,7). During an epidemic, several types are usually  
62 detected and replace each other over time depending on immunity of the population (2).

63 Mayotte island, located in southwestern Indian Ocean (figure S1), became a French  
64 department in 2011. It has a maritime tropical climate with a rainy season from November to  
65 May. Mayotte is an attractive destination for migrants from the area (Comoros and Madagascar)  
66 and in 2017 had 256,500 inhabitants, with an estimated population density around 690 per km<sup>2</sup>  
67 (10). The population of Mayotte is particularly young: 50%, 30%, 15%, 7% and 3% of the  
68 inhabitants are respectively under 18, 10, 5, 2 and 1 year old. Half inhabitants don't have French  
69 nationality and approximately 30% houses are not connected to collective sanitation plants (10).  
70 In Mayotte, only one study reported a large outbreak of EV infections associated with  
71 conjunctivitis. Although a total of 12,000 cases was estimated, only 3 clinical samples were  
72 collected for testing; EV genome was detected in 2/3 but not typed (11). Regarding CNS  
73 infections caused by EVs and PeVs, no data are available as the routine screening of  
74 cerebrospinal fluids (CSFs) of patients with suspected meningitis do not include EVs nor PeVs  
75 RT-PCRs. In Madagascar, 55 enterovirus strains were identified between 1994 and 2002 : 25 and  
76 30 isolates belonged respectively to human enterovirus B species and human enterovirus C  
77 species (12).

78 In this study, we aimed to assess the need for EV and PeV diagnosis in Mayotte.

79

## 80 **Methods**

### 81 *Population*

82 CSFs were collected from patients with suspected meningitis, admitted to Mayotte  
83 Central Hospital between March 1 and June 30, 2019. CSFs with a minimum volume of 200  $\mu$ l  
84 were retrospectively screened for EV and PeV by PCR. The CSFs were initially referred to the  
85 laboratory for biochemical and bacteriological screening and stored at -80°C. Clinical symptoms  
86 of patients for whom an EV or PeV meningitis was diagnosed based on positive genome  
87 detection in CSF were retrospectively collected from patients' clinical records, as well as the CSF  
88 cell count.

### 89 *Ethics*

90 This work was a retrospective non-interventional study with no addition to standard care  
91 procedure. The study was carried out in accordance with the ethical guidelines for medical  
92 research of the Mayotte Hospital and was approved by the institutional review boards of the  
93 Mayotte Hospital Center.

### 94 *EV and PeV genome detection*

95 EV and PeV RT-PCRs were performed in the laboratory of Mayotte Central Hospital.  
96 CSF were tested within a maximum of 223 days after sample collection. Total nucleic acid was  
97 extracted from 200  $\mu$ l of CSF with QIAamp Viral RNA Kit (Qiagen, Courtabœuf, France) and  
98 eluted in 50  $\mu$ l RNase-free water. We used previously described “in house” RT-PCR assays for  
99 EV (13) and PeV (14) genome detection on LightCycler 480 (Roche diagnostics). Some  
100 adjustments were implemented. After reverse transcription at 50 °C (5 min) and initial  
101 denaturation at 95 °C (20 seconds), amplification was performed in 40 cycles with a standard  
102 TaqMan two-step protocol (60 °C for 30 s and 95 °C for 5 s). Positives controls provided by the

103 coordinating laboratory of the National Reference Center (NRC) for EV and PeV (Lyon, France)  
104 were included to ensure quality of each experiment.

#### 105 *EV and PeV genotyping and phylogenetic analysis*

106 Positive EV CSFs or RNA extracts were sent to the associated laboratory of the NRC  
107 (Clermont-Ferrand, France) for genotyping. Identification of EV type was performed by  
108 amplification and Sanger sequencing of the partial VP1 coding region as previously described  
109 (15)(13). Virus identification was performed by BLAST analysis and confirmed by phylogenetic  
110 analysis (16)(14). To investigate the spatiotemporal relationships between the predominant  
111 strains collected in Mayotte and those circulating elsewhere, we compared the nucleotide VP1  
112 sequences assigned to the different EV types with homologous sequences available in public  
113 databases (as of May 28, 2021) and in the database of the associated laboratory of the NRC  
114 which prospectively collects and genotypes samples from 13 laboratories in France  
115 (<https://cnr.chu-clermontferrand.fr>). After elimination of redundant sequences, an alignment of  
116 127 E-16 sequences of 326 nucleotides were constructed. Phylogenetic relationships were  
117 inferred with the neighbor-joining method from distances estimated with the Tamura-Nei model  
118 of evolution implemented in MEGA6 software (<http://www.megasoftware.net>). The sequences  
119 determined in the study (n=10) were submitted to the EMBL database (accession numbers  
120 OK585074 – OK585083).

#### 121 *Statistics*

122 The p-value was computed using a Fisher's exact test with a 95% confidence interval to  
123 compare rates of tested and positive CSF per month.

124

125 **Results**

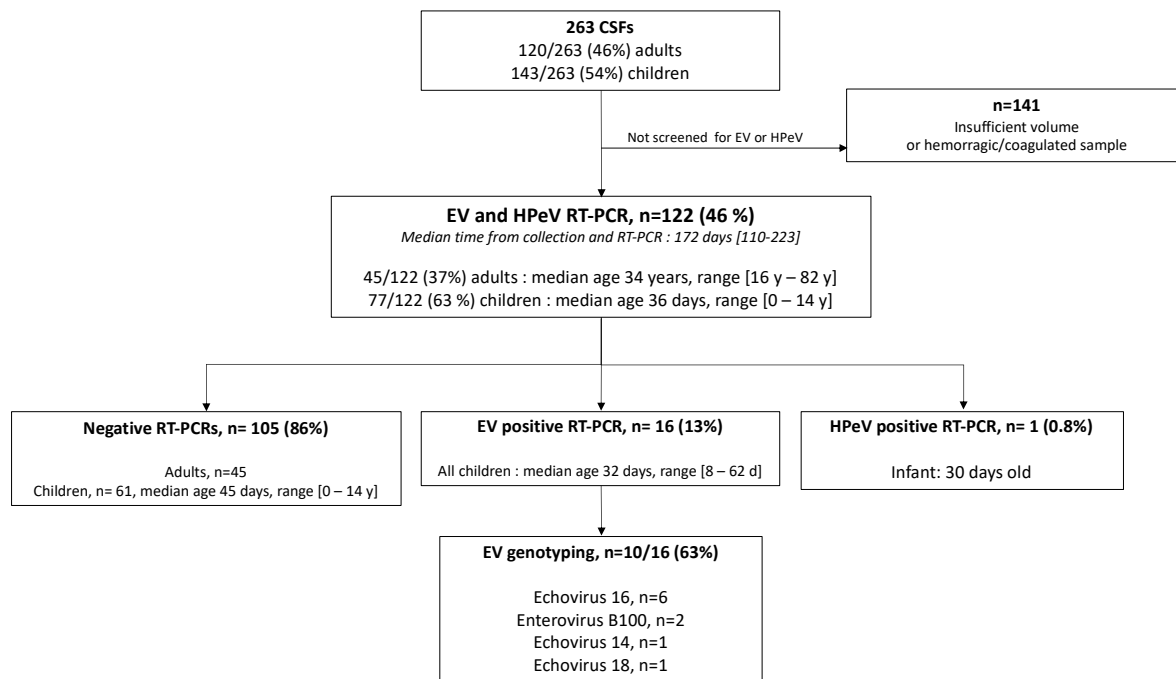
126 *Detection of EV and PeV Infections*

127 From March 1 to June 30, 2019, 263 CSFs were collected from 120 adults (46%) and 143  
128 children (54%) within the routine clinical management. Sufficient volume was available from  
129 only 122 specimens and 141 were hemorrhagic, coagulated or had insufficient volume. EV and  
130 PeV RT-PCR could be performed on 122/263 CSFs (46%) from 45 adults and 77 children  
131 among whom 32 were neonates (12 were 10 days old or less), 22 were infants less than 3  
132 months, and 8 infants less than 2 years old (figure 1). EV RT-PCRs were positive in 16/122  
133 cases (13%). The median age was 32 days (range: 8-62 days), and 41% (7/16) were neonates;  
134 56% were male. Fever was reported in 15/16 cases (94%), gastrointestinal disorders in 5/16  
135 (31%), respiratory signs in 3/16 (19%), rash in 2/16 (13%) and neurological disorders (neck  
136 stiffness and irritability) in 2/16 (12%) cases (Table S1). CSF cell counts showed a median of 4  
137 leukocytes/mm<sup>3</sup>. The number of EV positive samples were higher in May (26%, P = 0.0043;  
138 figure 2), with a positivity rate of 2.5% (1/40), 14% (4/28), 26% (9/34) and 10% (2/20) in March,  
139 April, May and June respectively (figure 2). One 51 days aged child was admitted in ICU for  
140 acute respiratory distress syndrome. PeV RT-PCR was positive in 1/122 (0,8%), CSF was  
141 collected in early June and only fever was reported on clinical examination of this 30-day-old  
142 child (Table S1).

143

144 Figure 1. Methodologic approach and results of screening and genotyping Enterovirus and  
145 Parechovirus in cerebrospinal fluids in Mayotte Island from March to June 2019. CSF,  
146 cerebrospinal fluid; EV, enterovirus; PeV, human parechovirus.





147

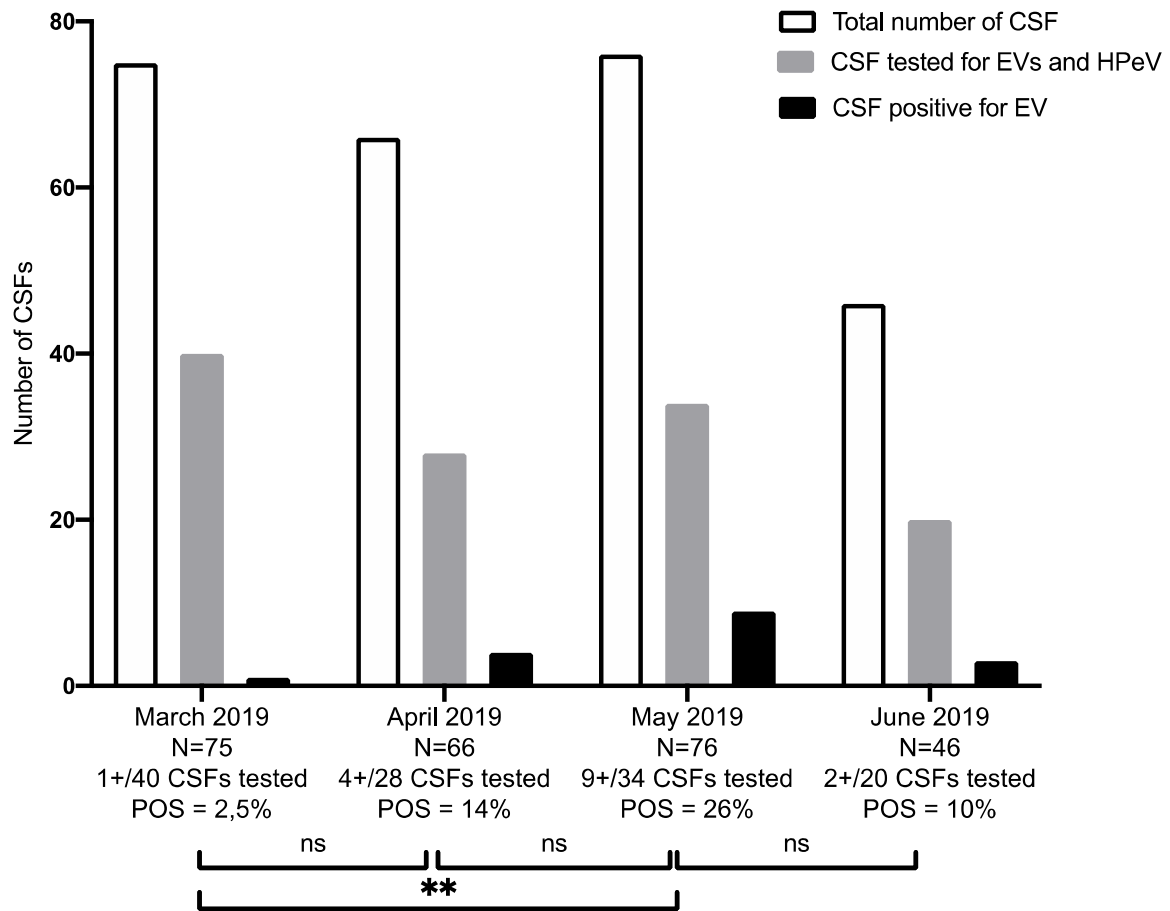
148

149 Figure 2. Number of cerebrospinal fluids tested per month and proportion of positive *Enterovirus*

150 RT-PCR in cerebrospinal fluid in Mayotte Island from March to June 2019. CSF, cerebrospinal

151 fluid; EV, enterovirus: PeV, Human parechovirus; POS, positive rate; \*\*, P=0.0043

152



153

154

155 *Genotyping and molecular epidemiology*

156 EV genotype was identified in 10/16 (62,5%) CSF of patients with proven EV infection.

157 Four different EV types were identified, all within the EV B species: Echovirus 16 (E-16, n=6)

158 was the predominant type, followed by EV-B100 (n=2), E-14 and E-18 (n=1, each) (figure 1).

159 No sample was available for genotyping in 3 cases and viral load was too low to perform

160 genotyping in 3 cases as estimated by threshold cycles values above 40 which could be due to

161 the time between sample collection and analysis and the shipping conditions between Mayotte

162 and metropolitan France. VP1 coding region obtained in this study were compared to

163 homologous sequences from Genbank and from the associated laboratory of the NRC. E-16  
164 sequences clustered in two different clades (bootstrap values >70%) I and II including sequences  
165 from strains isolated worldwide from 1991 to 2018 or in Europe (except one Chinese strain  
166 isolated in 2016) between 2003 and 2020, respectively (figure S2). All E-16 sequences  
167 characterized in this study clustered together in clade II with sequences of viruses isolated in the  
168 Netherlands, France and China between 2003 and 2016. Only 11 EV-B100 sequences from  
169 viruses isolated in India in 2007-2009 (n=6), Bangladesh (2000 and 2008, n=3) and Pakistan  
170 (2014, n=1) were available in databases which hampered any reliable phylogenetic analysis.  
171 Reconstruction of the phylogenetic trees for the E-18 or E-14 sequences did not allow to  
172 determine any clade; however, the E-18 sequence obtained in this study displayed close  
173 relationships with those of strains from USA in 2019 (data not shown).

174

## 175 **Discussion**

176 EVs are the main cause of acute aseptic meningitis in children and adults. Testing for EV  
177 genome in CSF is recommended in cases of low suspicion of bacterial meningitis (17) and in  
178 cases of suspected encephalitis (18). Moreover, obtaining an EV-positive CSF result usually  
179 reduces duration of hospitalization and allows antimicrobial treatment to be stopped (19).

180 In 2019, EV or PeV genome detection was not implemented in routine due to the lack of  
181 data on prevalence, the absence of specific treatment and the existence of local health  
182 authorities' priorities such as arthropod-borne microorganisms like dengue or malaria diagnosis.  
183 Consequently, no data about the epidemiology of EV or PeV infections in Mayotte is currently  
184 available.

185           This 4-months pilot study provides the first accurate data on the involvement of EV and  
186 PeV associated with neurological signs in Mayotte. CSFs were collected at the end of the rainy  
187 season when hospital visits for meningitis were increasing, as the number of EV infections cases  
188 could increase with temperature and relative humidity (5). Prevalence of EV meningitis was  
189 13%, which is less than reported in temperate countries in summer or in autumn (20,21). Even  
190 though this study provides partial epidemiological data, studies over longer time periods are  
191 needed to have a better picture of EV meningitis epidemiology in this specific French department  
192 located in the Indian Ocean and have an idea of strains circulating in the Comoros/ Madagascar  
193 region. All patients with EV meningitis were young infants aged of 8 to 62 days with a median  
194 of 32 days, which can be explained by the higher proportion of tested CSFs in infants less than 3  
195 months (70% of CSFs were collected from children). In France in 2019, children under 1 year  
196 old represented 41 % of patients with positive EV in CSF specimens (22); 22 % were neonates  
197 (< 30 days). In our study, 41 % of the patients were neonates. High prevalence in this age group  
198 could be associated with poor hygienic conditions in the newborn's environment, which would  
199 allow an earlier first contamination in life by fecal-oral transmission, for children who are not  
200 immune to enteroviruses yet. The lack of EV detection in adults may be due to a higher adult  
201 immunity acquired during childhood or mild symptoms in adults that do not require a hospital  
202 visit.

203           Only one PeV meningitis was diagnosed in a neonate. PeV infection are more frequently  
204 described in young infants (4,20), in our study the proportion of children under 3 months of age  
205 was 44% (54/122). PeV screening is thus recommended for infants under 1 year old presenting  
206 with sepsis-like disease or suspected meningitis. This low prevalence was also observed in  
207 France in 2019 (22) and could be explained by distinct epidemiological features of PeV

208 infections for which a biannual circulation of PeVs associated with meningitis is often reported  
209 in temperate countries in even years (4).

210 EV genotyping is required in 1) any severe neurological infection to detect EV known to  
211 be associated with encephalitis and myelitis (EV-A71 and EV-D68, for example) and to exclude  
212 a poliovirus infection, 2) in any other severe infection (neonatal infections, myocarditis,  
213 respiratory distress, sudden infant death syndrome), 3) to investigate cluster of EV infections  
214 (nosocomial infections) (22), and 4) to monitor circulation of EV types. Here, we report the first  
215 epidemiological overview of EV types detected in CNS infections in Mayotte. All 4 identified  
216 EV types belong to the enterovirus B species, which includes the types most frequently involved  
217 in meningitis. If E-16 was the predominant type in our study and is known to be associated with  
218 meningitis, it is not the most predominant in literature (23). However, in metropolitan France, the  
219 proportion of EV meningitis associated with this type increased from 0.6 % to 2.1 % between  
220 2017 and 2019 (22). Based on VP1 coding region, the E-16 circulating strains in Mayotte were  
221 genetically related to French strains isolated in 2018 and 2019. EV-B100 was detected in a 30-  
222 day-old child with fever and diarrhea and in a 26-day-old child with fever and mild neurological  
223 symptoms. This type has been isolated for the first time in Bangladesh in 2000 (1,24) through the  
224 surveillance of poliovirus eradication in the stool of a child presenting with acute flaccid  
225 paralysis (AFP). Since then, detection of EV-B100 was reported in 2007-2009 in India and in  
226 Bangladesh in stool of children with gastroenteritis or AFP and in 2014 in Pakistan in sewage  
227 water (25–28). E-14 and E-18 were each detected in one patient and are well known types  
228 associated with meningitis (1,20,29), especially E-18 which is often reported during EV  
229 meningitis outbreaks (22).

230 Our study has some limitations. Only 46% of CSFs were available for analysis.  
231 Newborns were over-represented compared to the rest of the population, among 77 children  
232 sampled 41% were newborns. In addition to CSF analysis, we did not perform EV RT-PCR on  
233 paired stool and respiratory specimens, which are also recommended in cases of meningitis or  
234 meningoencephalitis (30), so the prevalence of meningitis caused by enterovirus may be  
235 underestimated.

236 In conclusion, the EV/PeV overall prevalence of 14% reported in this pilot study in  
237 Mayotte highlights the importance of implementing this diagnosis which can impact the duration  
238 of hospitalization, the consumption of antimicrobial treatment, and thus reduce the risk of  
239 antimicrobial resistance (19) which is an important issue in the area according to the Indian  
240 Ocean Commission (31). Even though diagnosis and surveillance are the rule in Europe, this is  
241 not true for all parts of the world and our study provides the first data for the Indian Ocean  
242 region. Surveillance of circulating EV types in locations not usually represented in enterovirus  
243 surveillance studies is needed to have a more complete picture of the epidemiology of EV  
244 infections and to monitor more accurately the emergence of EV types that could be associated  
245 with distinct or severe clinical presentations.

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338 **Conflict of interest:** None declared

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340 **Funding statement:** None declared

341 **Authors' contributions:** CVF designed the research; LK performed lab tests; JF: performed lab  
342 tests, compiled, computed, and analyzed the data; AM and CH: performed genotyping,  
343 computed, and analyzed the data; CVF, LC and SO contributed to sample collection and  
344 supervision; LC, SO and CH contributed to data interpretation; JF, AM and JD wrote the  
345 manuscript. All authors reviewed the manuscript.

346 **Author Bio** Jacques Fourgeaud is a clinical virologist at the virology laboratory of Necker  
347 Children's Hospital (Paris, France). He is attached to the research unit EA 7328 PACT of  
348 Imagine Institute at the University of Paris. His research focuses on congenital CMV infections  
349 and clinical metagenomics in rare fetal and immunocompromised diseases.