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# Interaction of enterovirus A71 with the human blood-brain barrier: an *in vitro* study

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## INTRODUCTION

**Enterovirus A71 (EV-A71)** is an RNA non-polio enterovirus that belongs to the *Picornaviridae* family. This virus is implicated in epidemic disease of hand-foot-mouth disease (HFMD) mostly in infants and children, especially in Asian countries. In Europe since 2015, the emergence of a multi-recombinant C1-like EV-A71 (C1v2015) induced outbreak of **meningitis** and **brainstem encephalitis** in Spain (2016) and France (2016, 2018). Damage to the blood-brain barrier (BBB) could explain such increase in the severity of symptoms.

The BBB is the larger interface between blood and brain, very selective, with a microvasculature of 600 km. **Endothelial cells (ECs)** constitute the wall of brain capillaries and confer to the barrier its function by expression of many adherent and tight junction proteins (ZO-1, claudin-5), specific transporters (Glut-1) and efflux pumps (P-gp, BCRP). **Pericytes**, astrocytes end feet and neurons, which compose the neurovascular unit, surrounds ECs and influences them.

This work aimed to investigate the **passage of free EV-A71 strain** through a **multi-cellular *in vitro* model of BBB**.

## MATERIAL & METHODS

### Evaluation of the BBB integrity after free viral infection

#### Paracellular Permeability

Basal fluorescence was measured after incubation (1h) with a hydrophilic compound : Lucifer Yellow (LY).

#### Trans-Endothelial Electrical Resistance (TEER)

The higher the measured resistance, the stronger the tight junction.

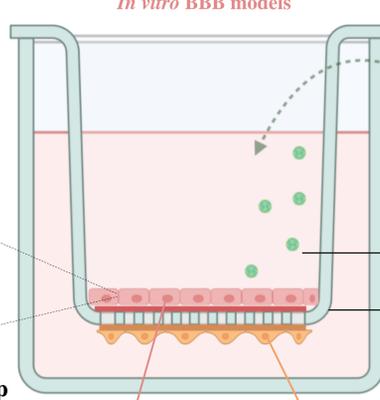
#### Immunofluorescence (IF)

Detection of tight junction protein zonula-occludens 1 (ZO-1).

#### Functional test : presence of efflux pump P-gp

Comparison of intracellular fluorescence of **Rhodamine 123** which naturally enter into EC, release by the efflux pumps P-gp, in the presence or not of a pump inhibitor (Elacridar).

#### *In vitro* BBB models



#### CD34+-derived endothelial cells

Cultivated on the apical side of Transwell™ and coating with Matrigel™

#### Bovine pericyte

Cultivated on the basal side of Transwell™ and coating with type I-rat collagen

Two EV-A71 strain were tested : **C1/16** and **C4/04** (MOI =1), and **echovirus 6 (E6)** was used as a positive control.

#### Inflammatory environment (TNF-α)

BBB was exposed to TNF-α (10 ng/ml)

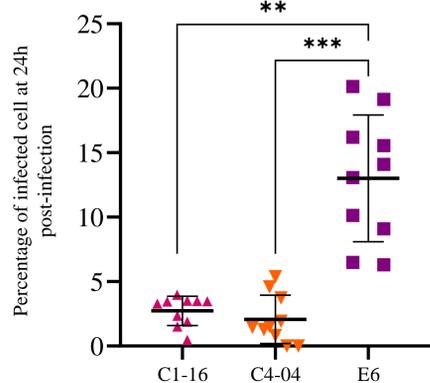
Luminal and basal supernatant were analysed at different time post-infection (pi)

- Presence of **infectious virus** : Titration
- Presence of **viral RNA** :
  - RT-PCR
  - Immunofluorescence

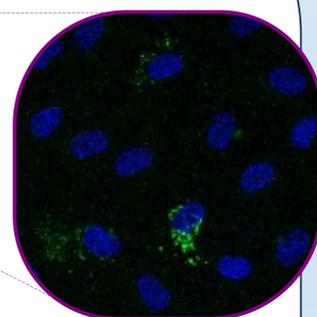
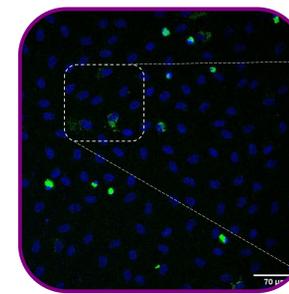
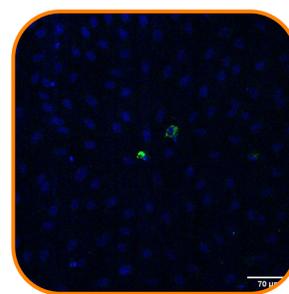
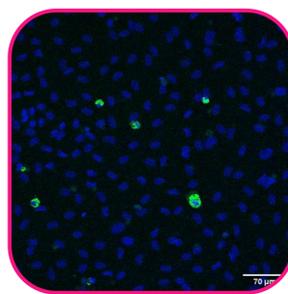
Detection of **viral RNA replication** (green). Infection efficiency was evaluated by IF.

## RESULTS

**Fig. 1** Percentage of infected cells at 24h pi



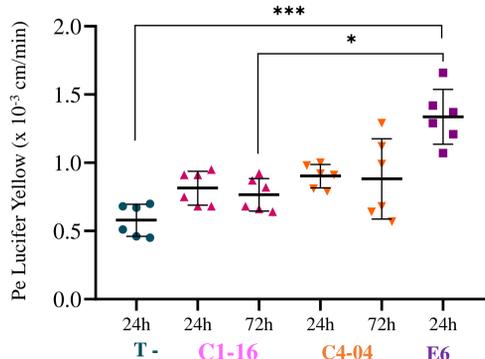
Percentage of infected cells realized from 10 confocal microscopy images.



Green = db RNA  
Blue = nuclei

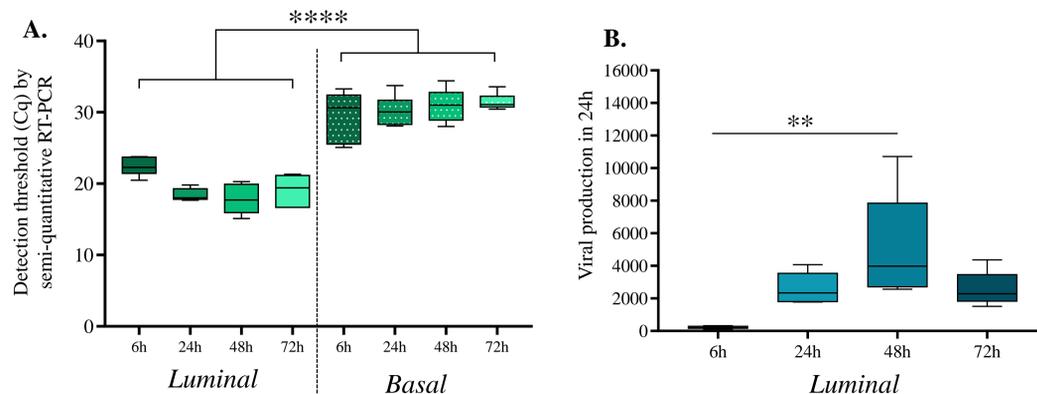
Endothelial cell-CD34+ are **weakly sensitive to EV-A71**: at 24h pi, less than 5% of cells are infected; in contrast, 13% of cells are infected with the E6 positive control.

**Fig. 2** Endothelial paracellular permeability after 24h and 72h post-infection



An increase of permeability is observed 24h pi between the positive control and negative control. **No difference** is observed between the negative control and EV-A71 infections.

**Fig. 3** Supernatants RT-PCR (A) and viral titration (B) in supernatants at different time post-infection (EV-A71 C1-16)

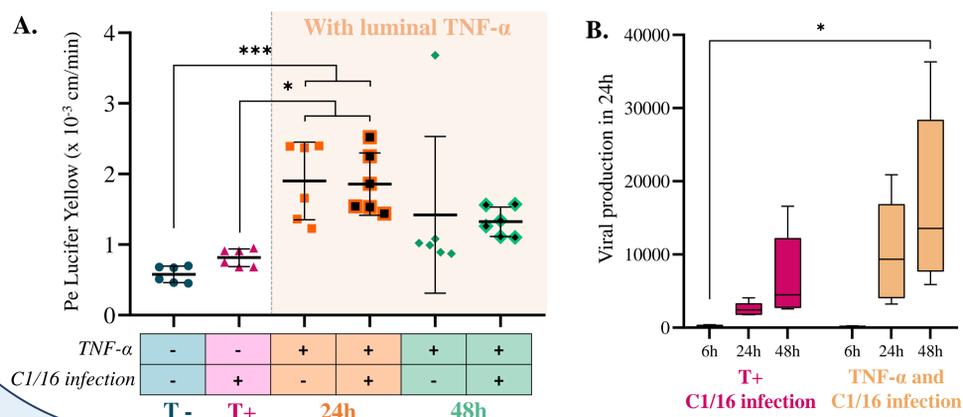


**Virus yield:** see Fig.3.B

**Virus yield:** not quantifiable (< 10<sup>6</sup>)

**Higher amounts of viral RNA** is detected in the **luminal side** than in the **basal side** (Fig 3.A). The yield of infectious virus was quantifiable only in the luminal side (Fig 3.B). A moderate amount was produced : less than 10,000 infectious particles at 48h pi. Of note, infectious virus is detected in small amount **in the basal side**.

**Fig. 4** Endothelial paracellular permeability (A) and viral titration (B) in an inflammatory environment with/without C1/16 infection



The TNF-α treatment increases the permeability at 24h **with or without infection** (Fig 4.A). The infectious virus is quantifiable only in the **luminal side** (below 40,000 infectious particles) (Fig 4.B).

\* p < 0,05  
\*\* p < 0,01  
\*\*\* p < 0,001  
\*\*\*\* p < 0,0001

## CONCLUSION

The **integrity** of the **BBB-like model** generated *in vitro* by a co-culture of CD34+-derived ECs and pericytes displayed **limited alteration** after **EV-A71 infection**. Results provide evidence of a **low viral replication** in ECs and **limited crossing** through the *in vitro* BBB model even in an inflammatory environment. Future investigations will focus on the **transmigration** of EV-A71 through infected **leukocytes**.

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