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# Development of 3D cellular models of infection of the human and equine central nervous systems by *Flaviviruses*.

Noémie BERRY<sup>1,\*</sup>, Valentine CHAILLOT<sup>1,2,\*</sup>, Aurélie MAGNIEZ<sup>1</sup>, Théo LA ROSA<sup>3</sup>, Hélène HUET<sup>1</sup>, Kamila GORNA<sup>1</sup>, Aïcha DEMBELE<sup>1</sup>, François PIUMI<sup>1</sup>, Bertrand PAIN<sup>3,&</sup>, Muriel COULPIER<sup>1,&</sup>

<sup>1</sup>UMR 1161 Virologie, Anses (Laboratoire de Santé Animale), INRAE, Ecole Nationale Vétérinaire d'Alfort, Université Paris-Est, Maisons-Alfort ; <sup>2</sup>Université Paris-Saclay, Gif-sur-Yvette ; <sup>3</sup>INRAE, USC 1361, SBRI, Lyon  
\*,&: equal participation ; corresponding author: [muriel.couplier@vet-alfort.fr](mailto:muriel.couplier@vet-alfort.fr)

## SUMMARY

**Tick-Borne Encephalitis virus (TBEV)** and **West Nile virus (WNV)** are two *Flaviviruses* that can cause severe and potentially fatal neurological disorders in human and/or horses, for which there is currently no antiviral treatment. These viruses have mostly been studied in cell lines, often not representative of their natural tropism. In order to have more physiologically relevant models, we recently used human neuronal/glia cells in culture and demonstrated their permissivity to TBEV and WNV (see poster François Piumi).

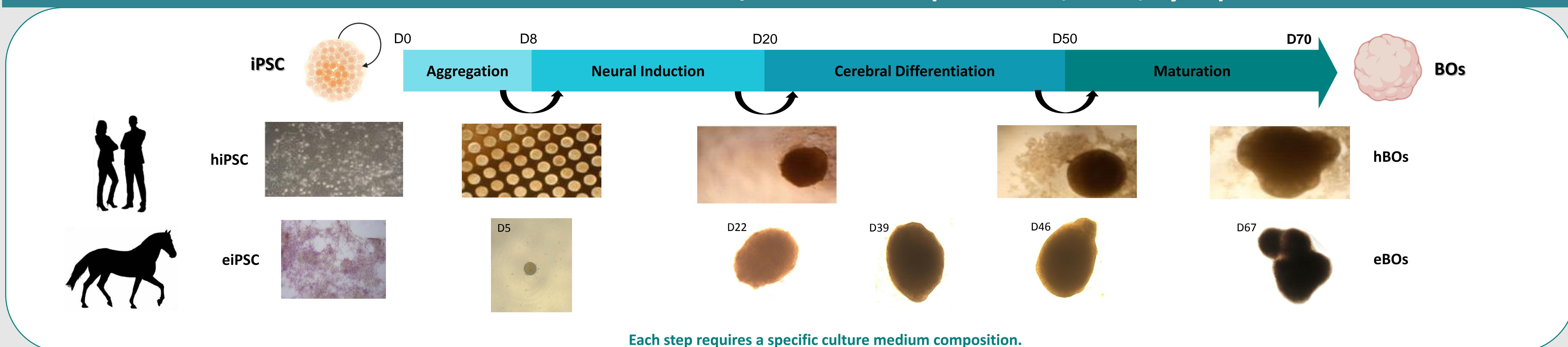
Here, with the aim of continuing to develop ever more relevant *in vitro* models, we produced and characterized **three-dimensional (3D) cellular models, called Brain Organoids (BOs)**, which mimic the tissue organization of the human and equine central nervous system.

We infected **human BOs (hBOs)** with TBEV and WNV and **equine BOs (eBOs)** with WNV and assessed viral replication through quantification of viral RNA (RT-qPCR) and viral particles (TCID 50) in supernatants, and immunostaining with antibodies specific to the viruses on 10 µm sections. Both viruses replicated efficiently in hBOs, as did WNV in eBOs.

Infected hBOs and eBOs were next used to test the **antiviral activity of chemical compounds**, some of which having a dose-dependent activity.

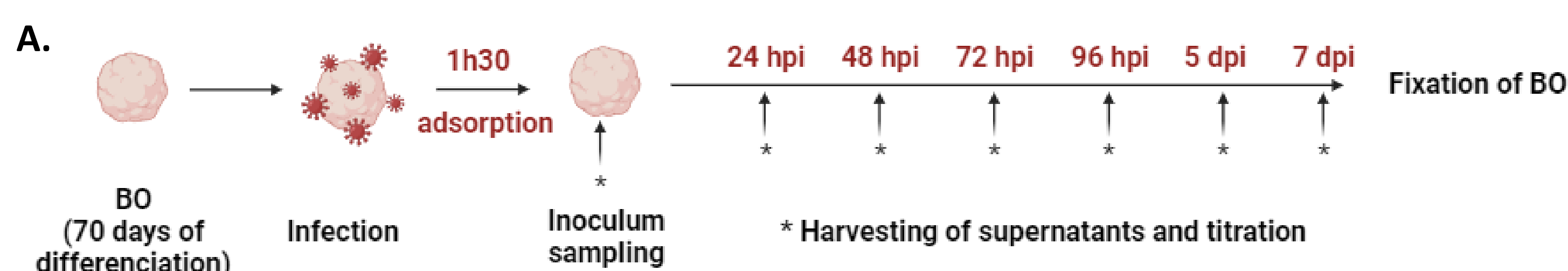
Thus, human and equine BOs represent novel, physiologically relevant, 3D *in vitro* models of viral infection which can be used to confirm antiviral activity of chemical compounds before *in vivo* testing.

## GENERATION OF OF HUMAN/EQUINE BOs (Dr B Pain, SBRI, Lyon)

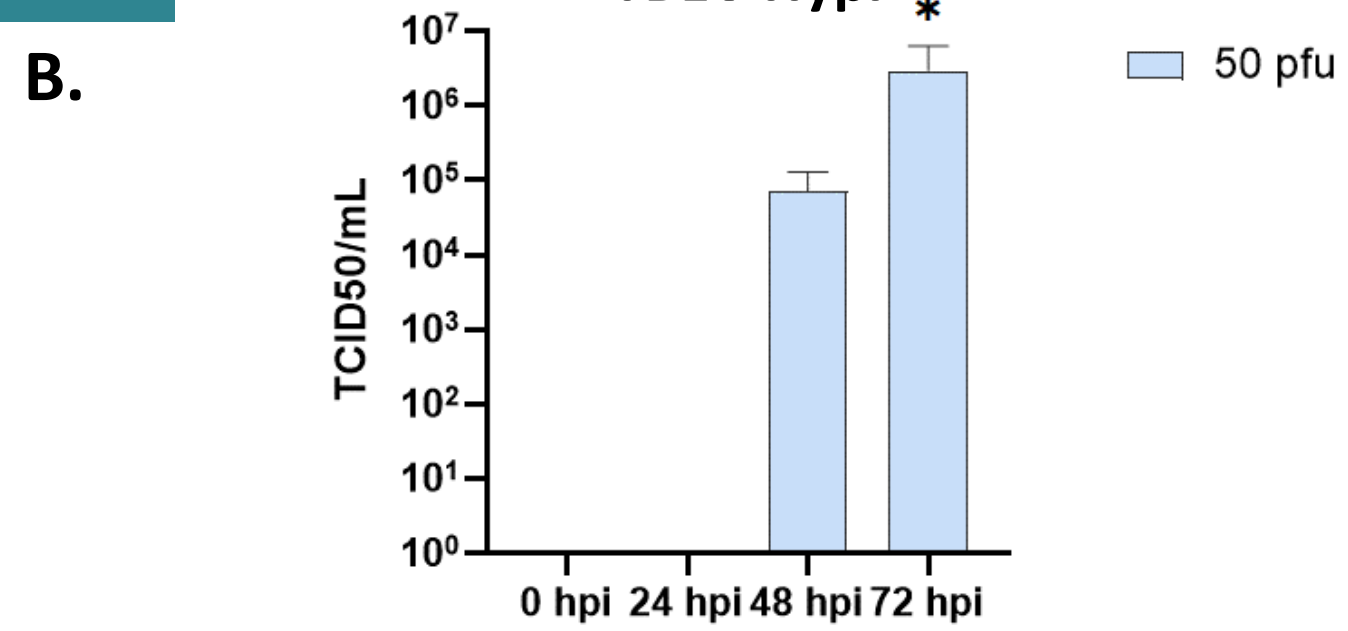


## BOs: 3D MODELS OF FLAVIVIRUSES INFECTIONS

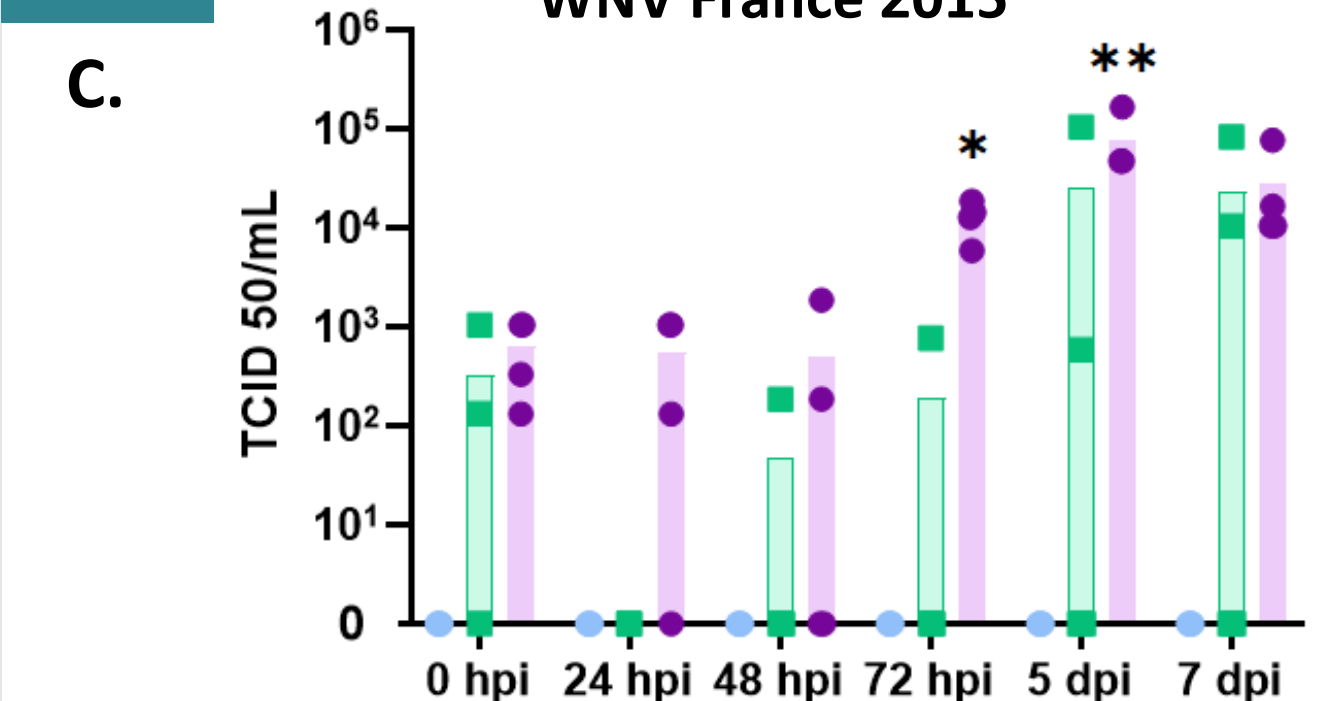
• Are BOs permissive to TBEV or WNV infection?



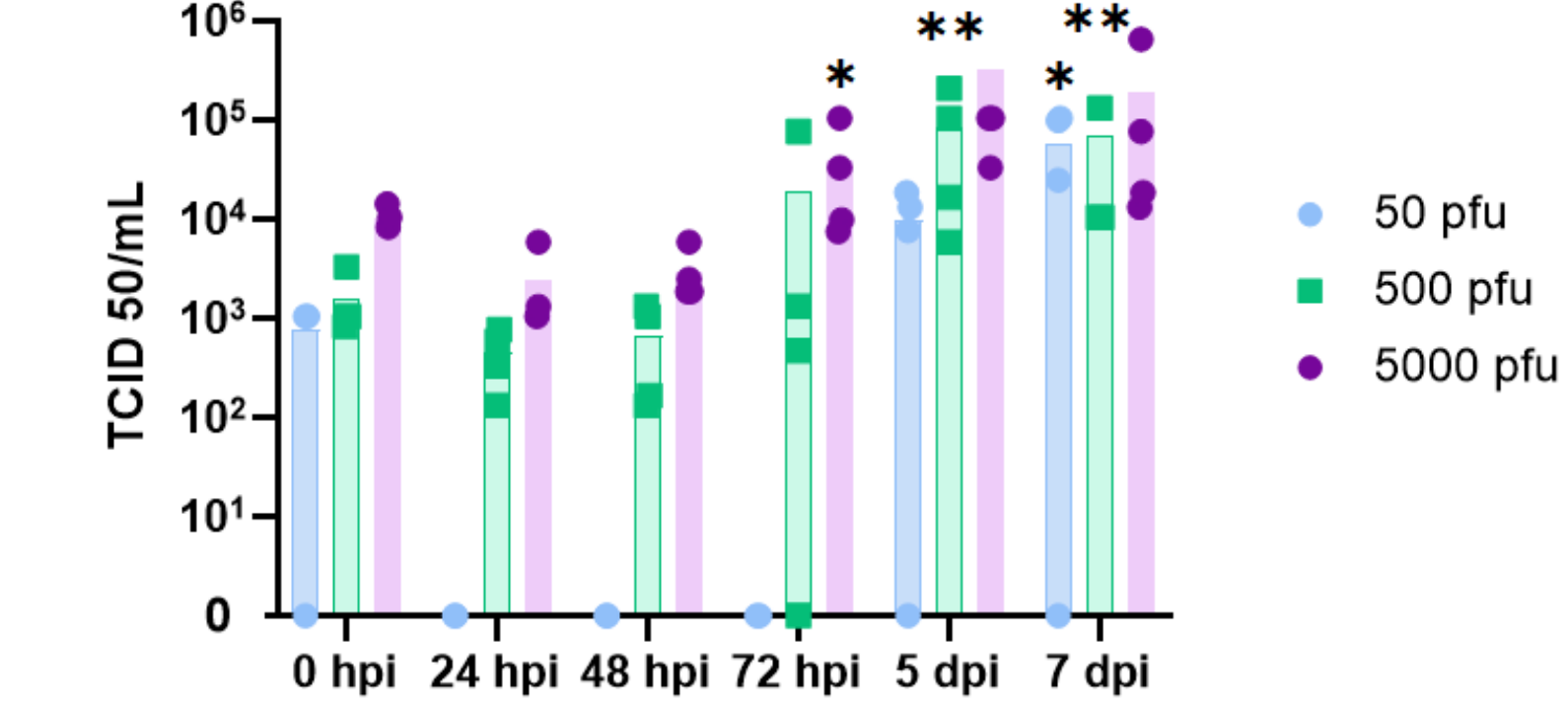
**hBOs**



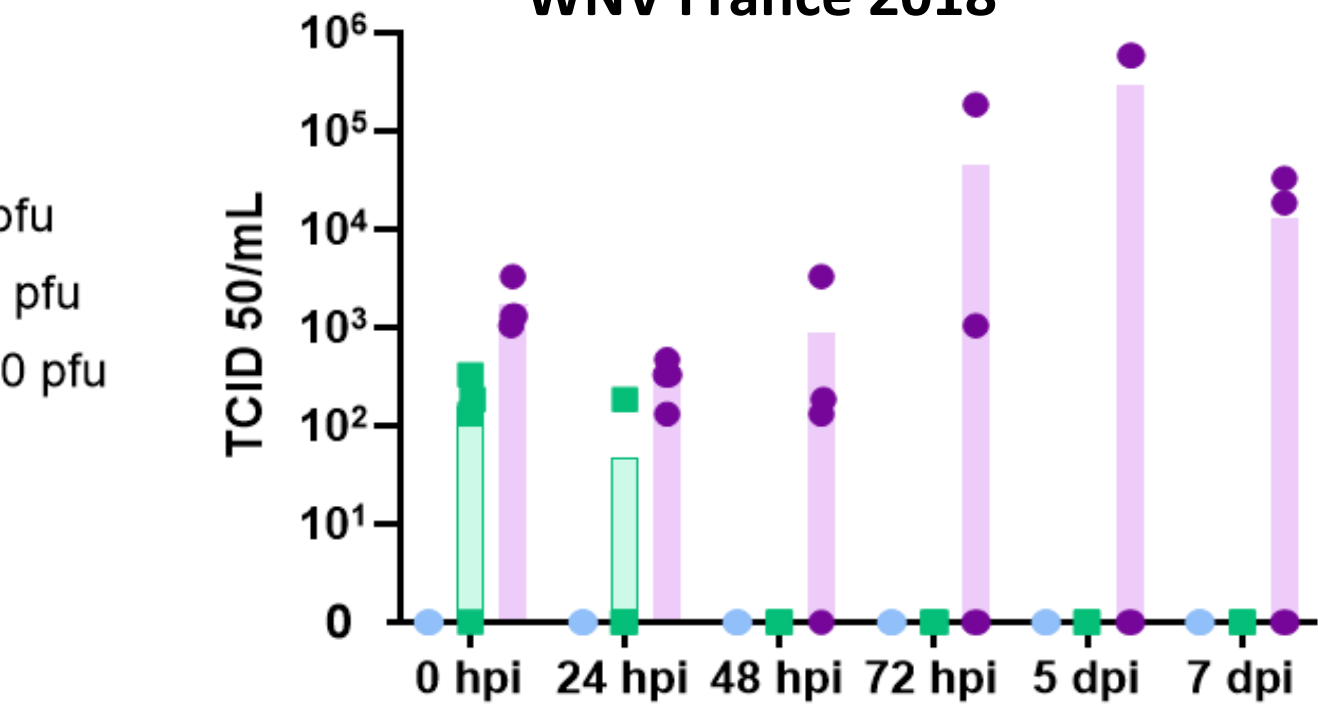
**hBOs**



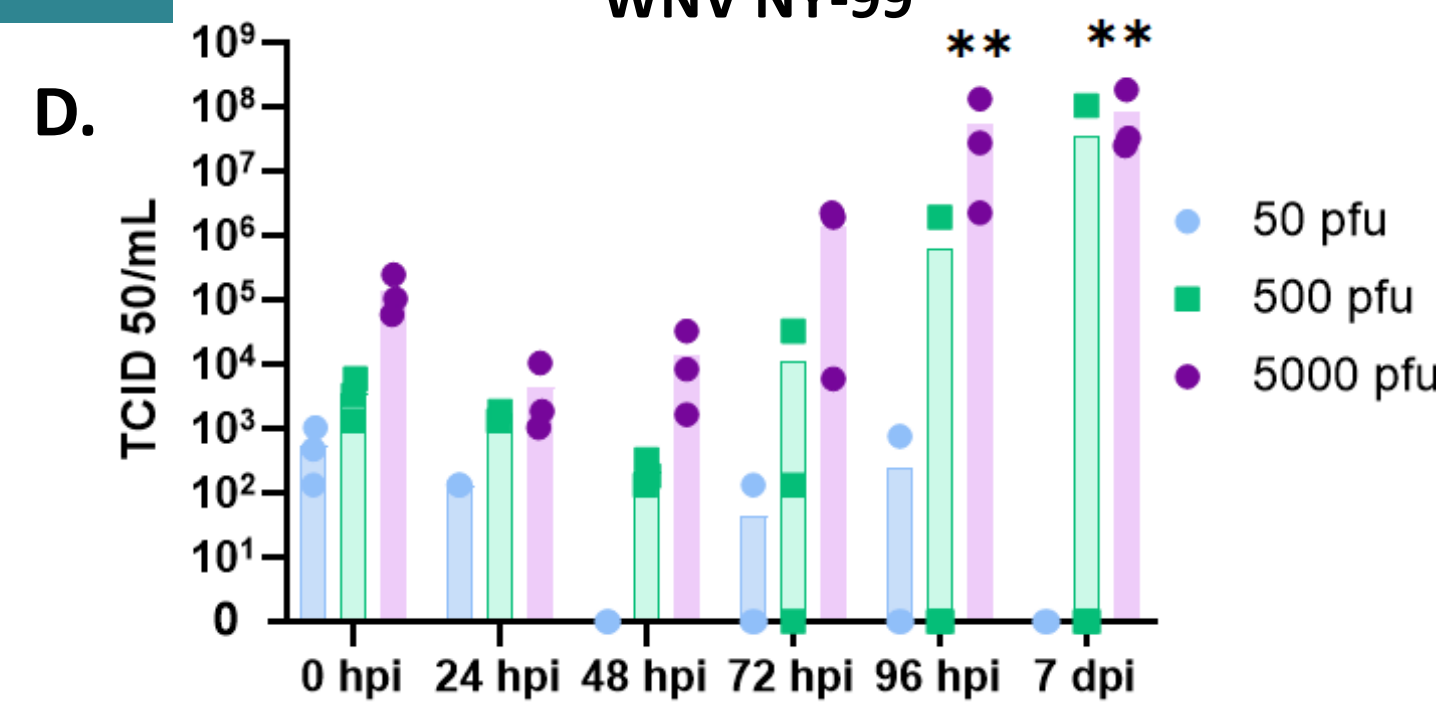
**hBOs**



**hBOs**



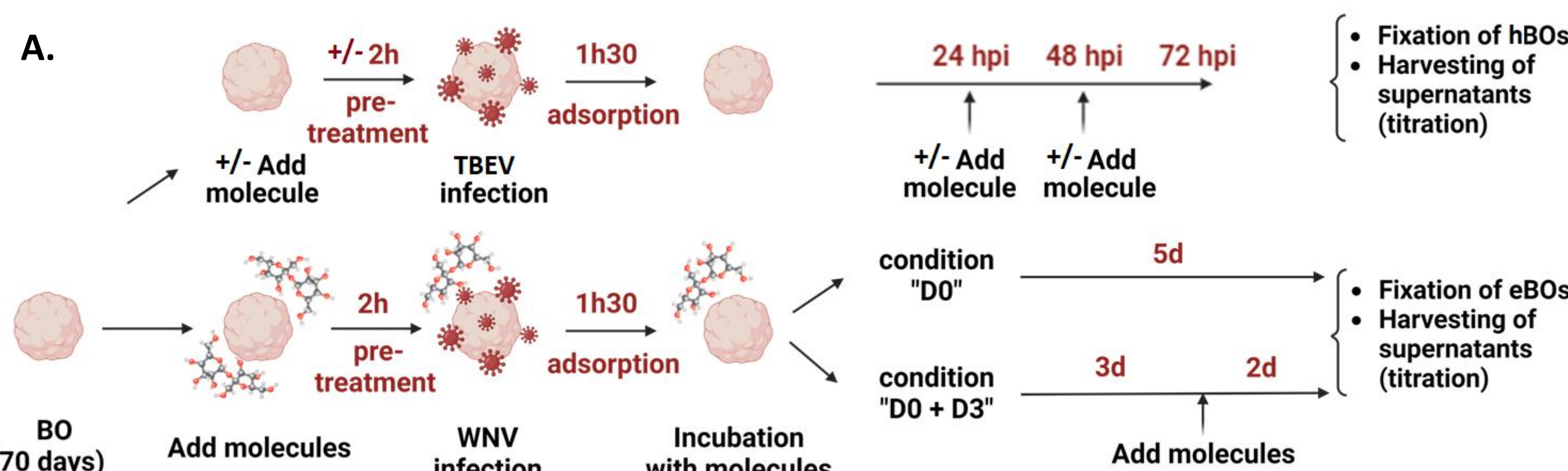
**eBOs**



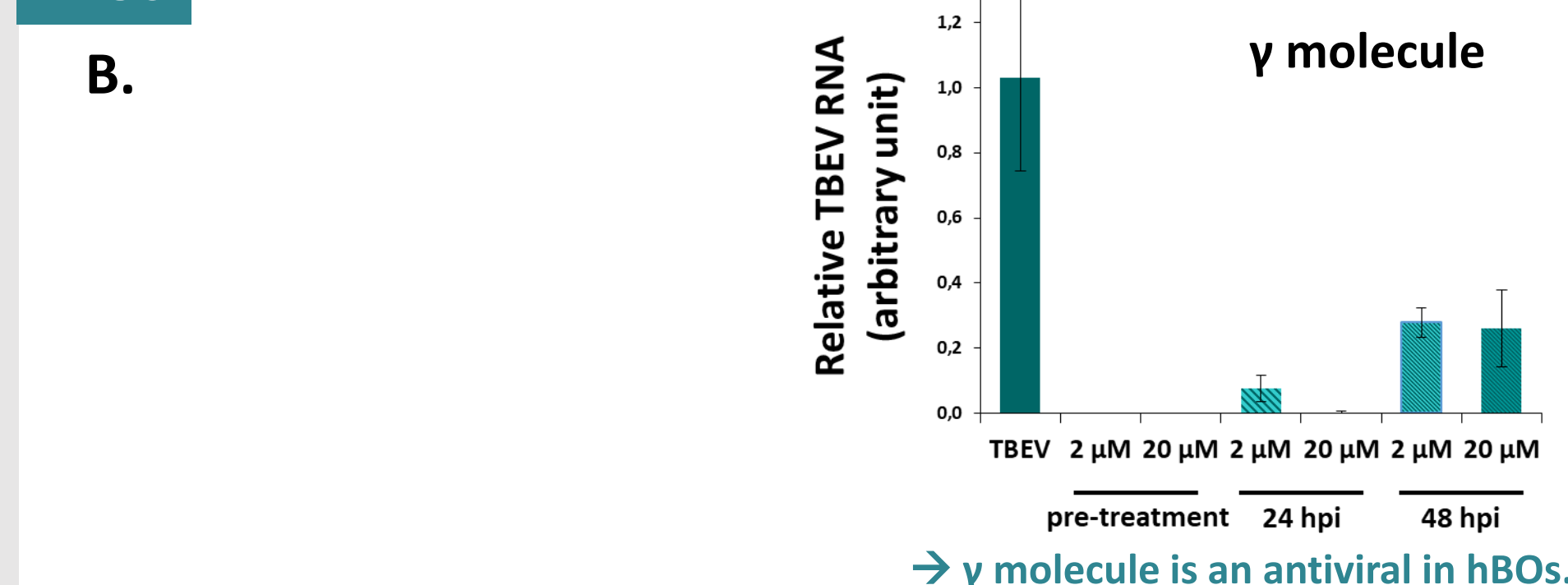
**A. Method for quantification of WNV replication in human and equine BOs.** Brain organoids were infected with 50, 500 or 5000 pfu of WNV or TBEV and culture supernatants were collected after 1h30 (0 hpi), 24 hpi, 48 hpi, 72 hpi, 96 hpi, 5 dpi or 7 dpi.  
**B-D. Quantification of viral particles in BOs supernatants by TCID 50.** B. hBOs infected with TBEV. Values plotted are the mean ± SD. C. hBOs infected with 3 strains of WNV. Histograms are the mean, dots the individual values. D. eBOs infected with WNV NY-99. Histograms are the mean, dots the individual values. \*: p-value < 0.05 ; \*\*: p-value < 0.01

## USING BOs TO IDENTIFY ANTIVIRAL MOLECULES

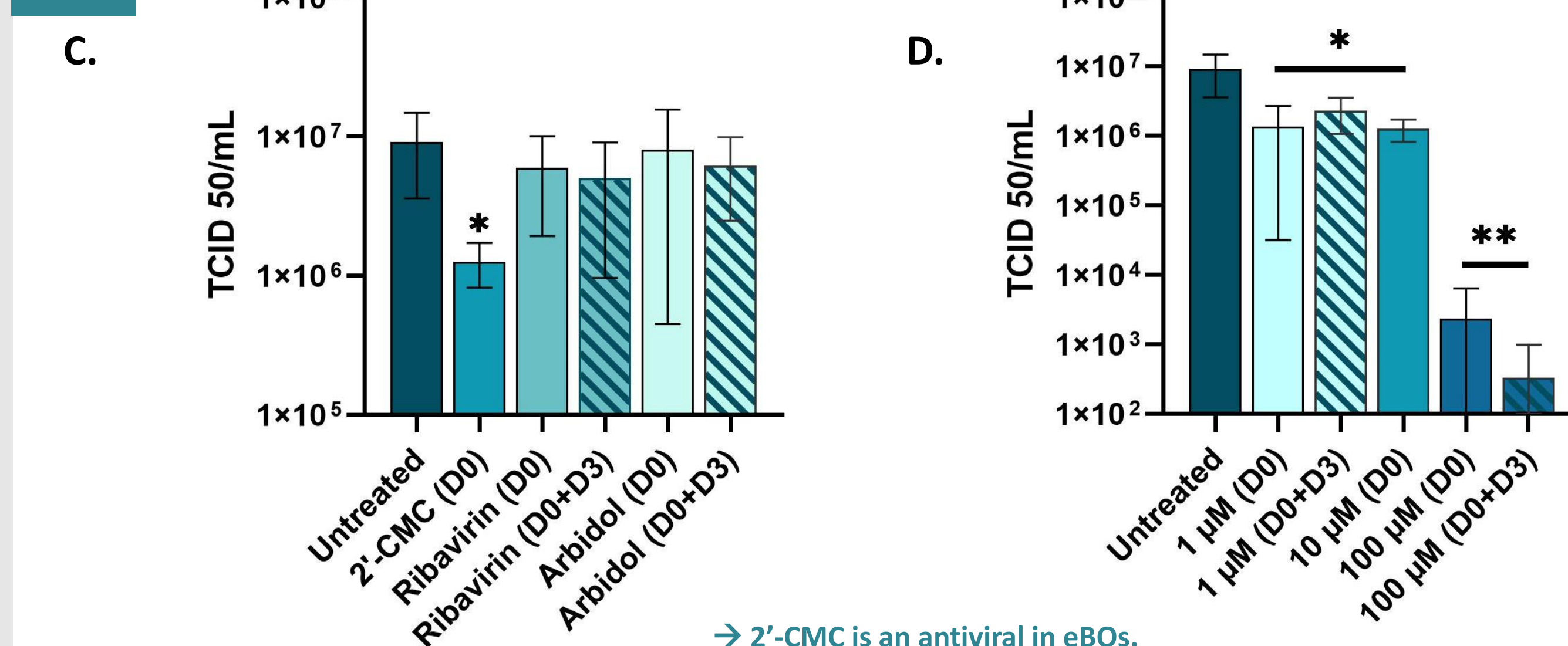
• Can BOs be used to test antiviral potential of molecules?



**hBOs**



**eBOs**



**A. Method for testing antiviral activity of molecules against TBEV and WNV in hBOs and eBOs.** After +/- pre-treatment with molecules, hBOs were infected with 50 pfu of TBEV and incubated for 3 days before fixation. Molecules were added before the infection (pre-treatment) or at 24 hpi or at 48 hpi. After pre-treatment with molecules, eBOs were infected with 5000 pfu of WNV and incubated for 5 days before fixation. Molecules were added again after 72 hours in some eBOs. Antiviral activity was assessed by quantification of relative viral RNA in supernatants by RT-qPCR and by quantification of viral particles in supernatants (TCID 50).

**B. Dose-effect of γ molecule antiviral activity.** γ molecule was added at a concentration of 2 or 20 µM, at pre-treatment or at 24 hpi or at 48 hpi in hBOs supernatants.

**C. Testing of the antiviral activity of three molecules.** 2'-CMC, ribavirin or arbidol were added at a concentration of 10 µM once (D0) or twice (D0+D3) in eBOs supernatants.

**D. Dose-effect of 2'-CMC antiviral activity.** 2'-CMC was added at a concentration of 1, 10 or 100 µM, once (D0) or twice (D0+D3) in eBOs supernatants. Histograms are the mean ± SD. \*: p-value < 0.05 ; \*\*: p-value < 0.01

## TAKE-HOME MESSAGES

- Identification of antiviral compounds with a high predictive value of therapeutic efficiency *in vivo* may require relevant 2D/3D *in vitro* pathological models.
- In order to mimic the tissue organization of the brain, 3D BOs structures (made of cells expressing early and late cortical markers) can be used.
- hBOs are permissive to TBEV and WNV ; eBOs are permissive to WNV.
- hBOs and eBOs can be used to validate the potential of chemical compounds as antivirals.
- One molecule already used in human medicine for other indications displayed antiviral activity against TBEV in hBOs.
- 2'CMC displayed antiviral activity against WNV in eBOs.