

# Variability in Ranavirus Infection in Cell Lines of Cold-Blooded Vertebrates

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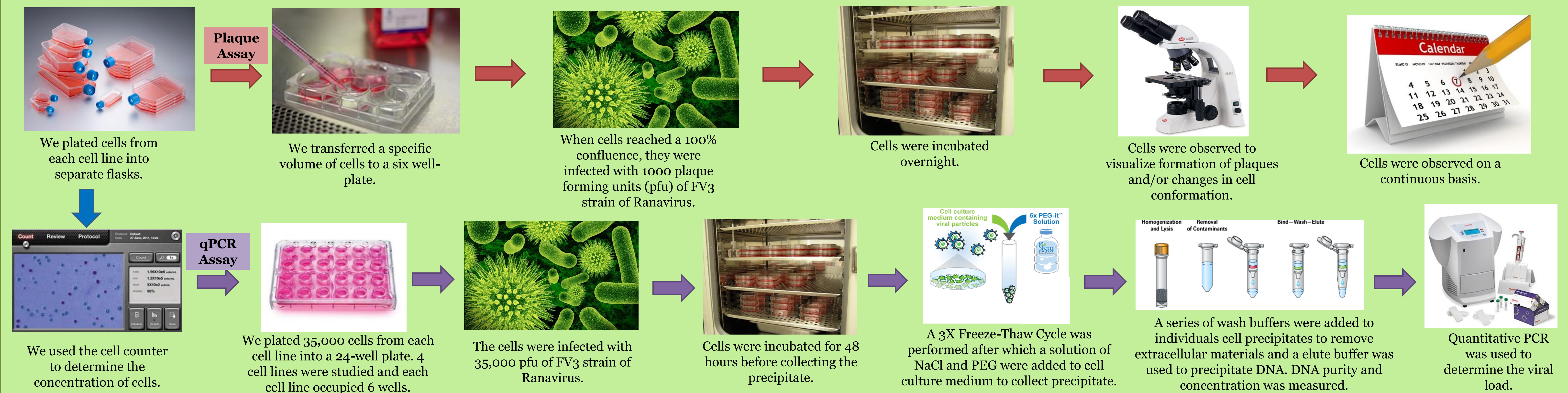
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## Abstract

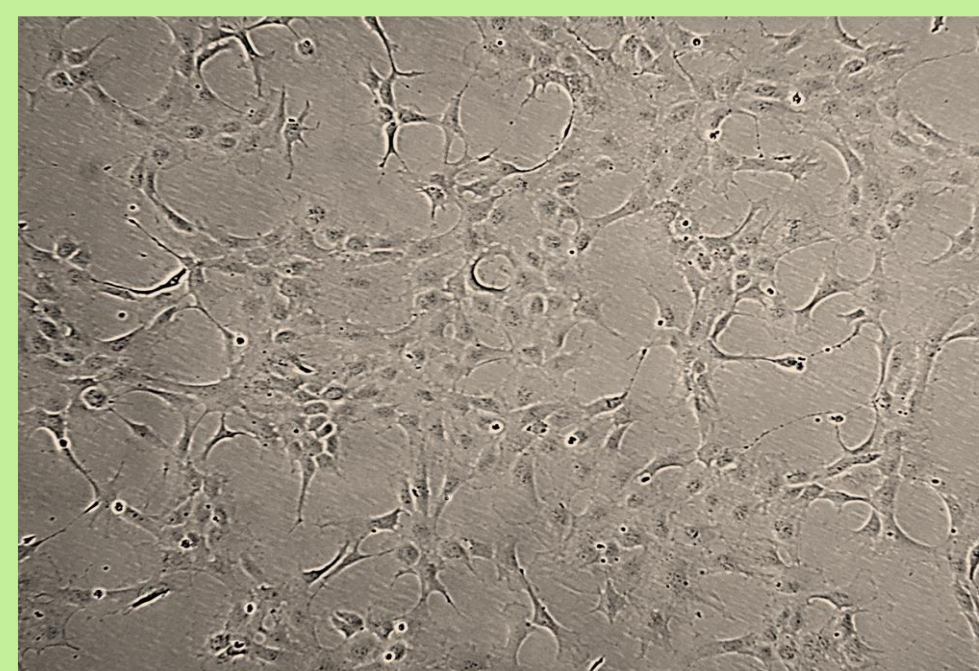
Ranaviruses are large, double-stranded DNA viruses that infect cold-blooded vertebrates, such as amphibians, reptiles, and bony fish. The spread of these viruses has been implicated as a contributing factor in amphibian population declines in the United States, Canada, and the United Kingdom. They are suspected factors in population declines of reptiles and fish and studies to evaluate this are ongoing. Understanding the susceptibility of different species to infection is an important step towards preventing viral spread, and therefore, reducing the impact of infection. The present study compares Ranavirus infection in cell lines derived from different host species. Cell lines from fish, Fathead Minnow and Epithelioma Papulosum Cyprini, amphibian, Xenopus Laevis, and reptile, Terrapene Carolina and Vipera Russellii, were used. Cell lines were infected with Frog Virus 3 strain because this is the most well-studied strain of Ranavirus. Multiple assays were used to determine the level of infectivity among the different host species. A plaque assay was performed with each cell line to determine if infection led to plaque formation, which is indicative of lytic replication. Periodic observation of infected cells monitored the development of plaques in each cell line and was used to estimate time for plaque formation. Additionally, viral load was measured by qPCR after infection of each cell type. Determining differences in viral load allows us to make inferences about alterations in the mechanisms of virus replication. Overall, these studies help us understand how the virus infects different species and the susceptibility of each to Ranavirus.

## Methods



## Cell Culture Background

### Fathead Minnow (FHM)



Epithelial cell type found in connective tissue and muscle



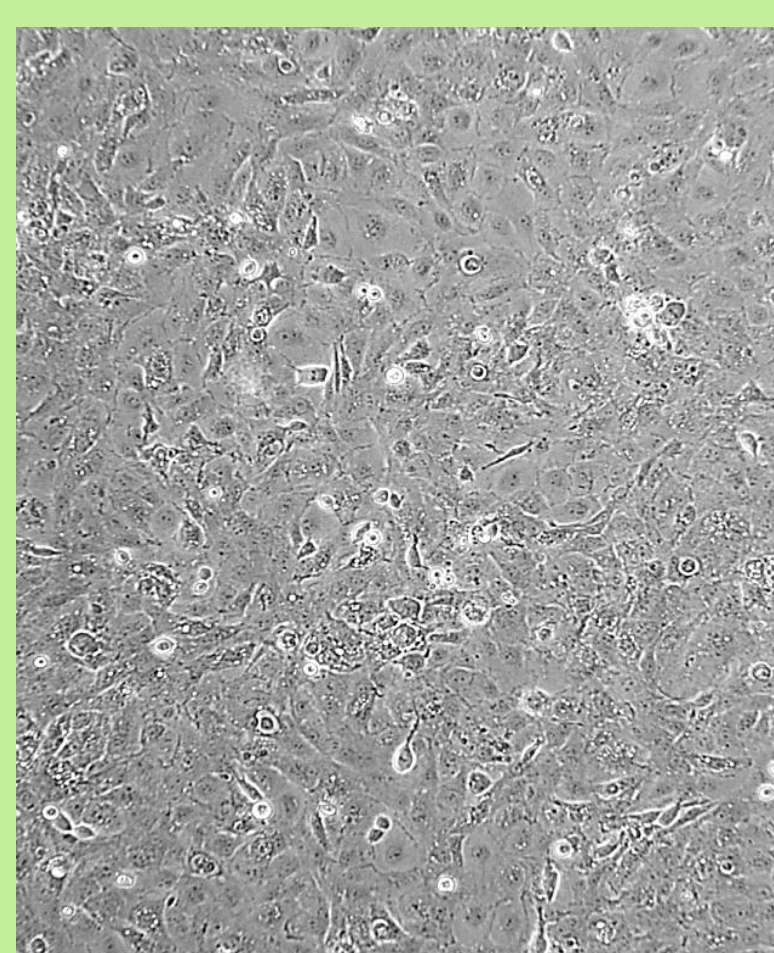
Species of temperate freshwater fish belonging to Pimephales genus of the cyprinid family.

### Epithelioma Papulosum Cyprini (EPC)



Epithelial cell type found in skin.

### Russell's viper (VH)



Cell type found in the heart.



It is a monotypic genus of venomous Old World viper. It is found in Asia throughout Indian subcontinent.

### Xenopus laevis, African Clawed Frog (A6)



Epithelial cell type found in the kidney.



It is a flat bodied, aquatic frog that is found in streams and ponds.

### Terrapene heart (TH-1)

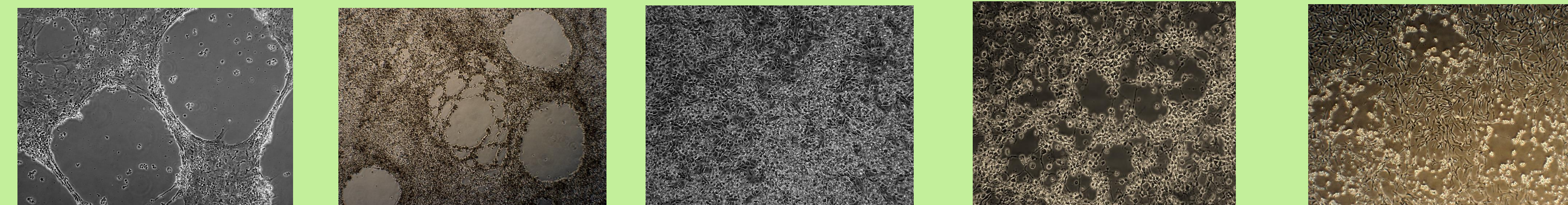


Epithelial cell type found in the heart.



It is a species of a box turtle; Terrapene carolina, that has a hinged lower shell that allows itself to enclose itself.

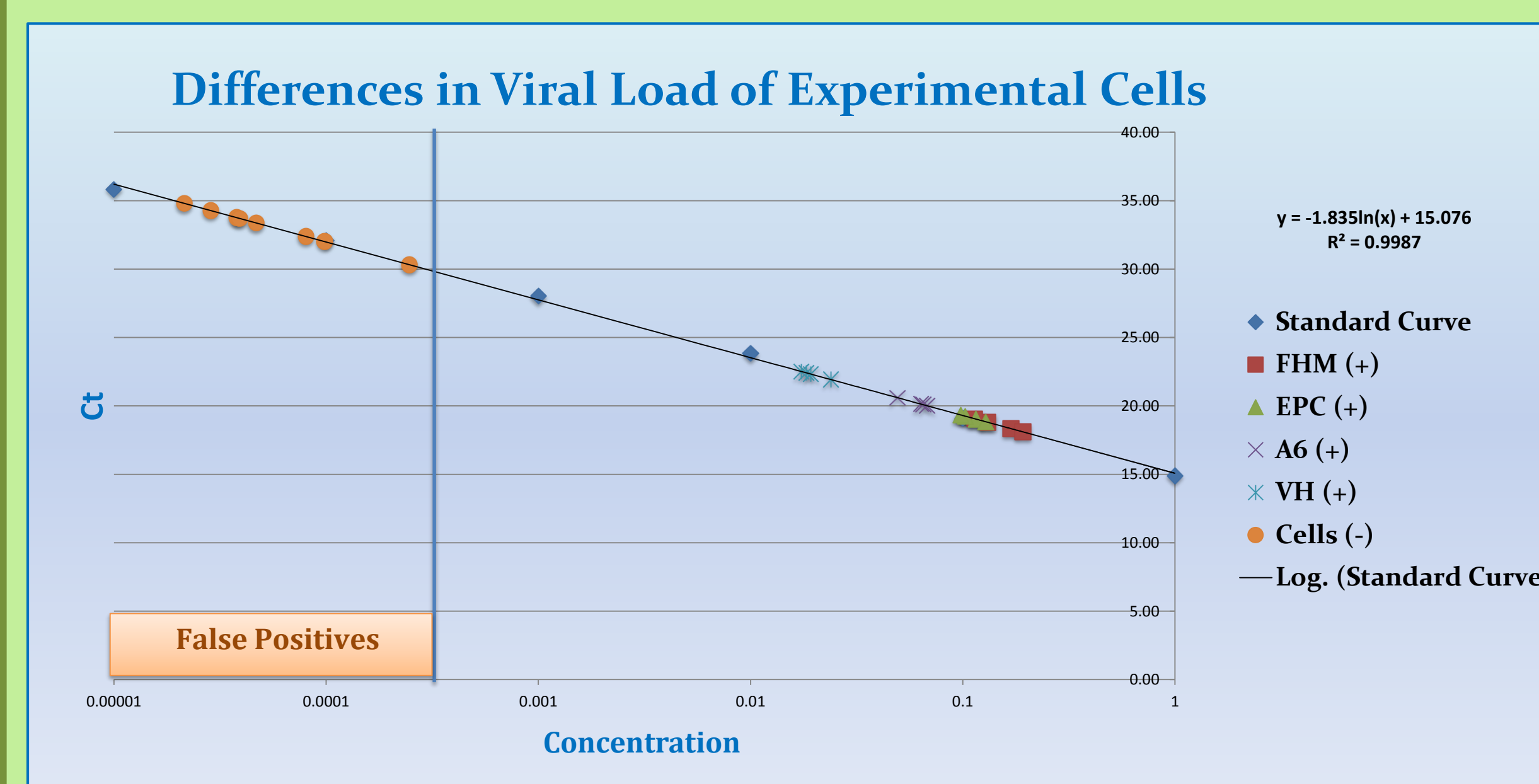
## Plaque Assays



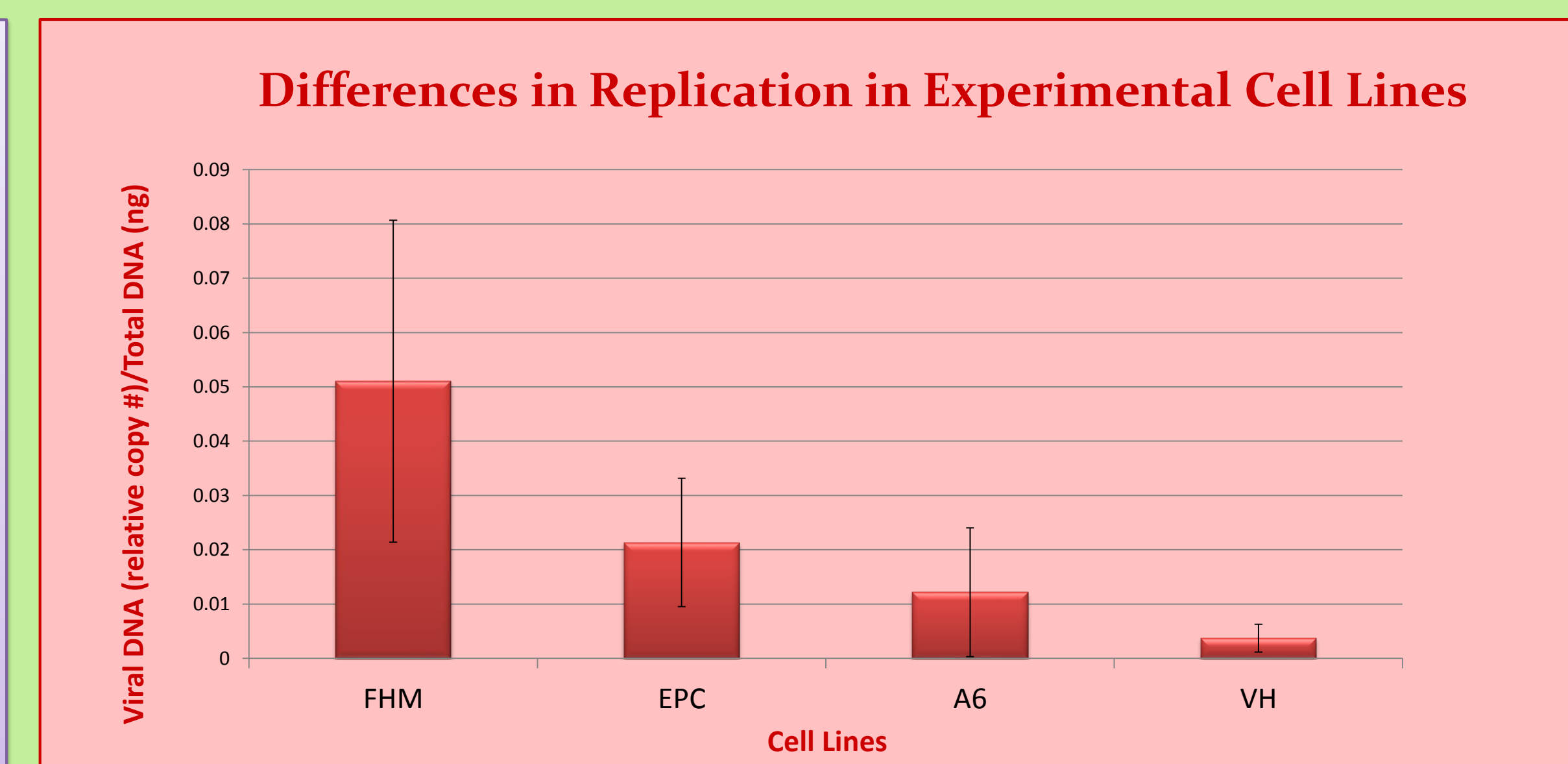
Cell Lines	FHM	EPC	A6	VH	TH-1
1	0-10 plaques	0-10 plaques	0-10 plaques	0-10 plaques	0-10 plaques
3	10-40 plaques	40-60 plaques	0-10 plaques	0-10 plaques	N/O
4	90-150 plaques	Cells dead	0-10 plaques	0-10 plaques	10-40 plaques
6	90-150 plaques	↓	0-10 plaques	10-40 plaques	150-250 plaques
7	90-150 plaques		0-10 plaques	40-60 plaques	150-250 plaques
9	Cells dead	↓	0-10 plaques	60-90 plaques	Cells dead
11	↓		0-10 plaques	Cells dead	↓

Observations were made on the number of plaques (shown in the table) and general size and shape of plaques (shown in images). Other than that, cell death was also noted for individual cell types. From the table, it is seen that FHM had the maximum number of plaques in the range of 90-150 before the cells died 40-60 plaques were observed in EPC cells before their demise. The A6 as very unique because no plaques were seen nor did cell death occur. VH cells did not exhibit plaque formation until Day 6 post-infection and 60-90 plaques were seen before cells died. TH-1 exhibited plaque formation on Day 4 and 150-250 plaques were seen before cell death occurred. The general plaque shape and sizes are shown in the images for each cell type. FHM cells had the largest plaque size, plaques in EPC cells were large and moderate, while the plaque size for VH and TH-1 cells were small.

## qPCR Results



There are a range of differences in the relative copy number of viral DNA between cell lines. The FHM cells contain the largest amount of viral DNA, while VH cells contain the smallest amount of viral DNA. This illustrates that the viral replication mechanisms differ between the cell lines. There was a very similar amount of viral DNA in FHM and EPC cells. This is evidence that the Fathead Minnow species (FHM and EPC cells) is more susceptible to viral infection by the FV3 strain of Ranavirus than Xenopus Laevis (Amphibian) or Russell's Viper (Reptile).



At 48 hours, FHM cells have the largest amount of viral DNA. There is a 14-fold difference between the relative copy number of viral DNA in FHM and VH cells. FHM and A6 cells differ moderately as there is a 4-fold difference in relative copy number of viral DNA between the cell lines. In contrast, there is only a 2-fold difference between FHM and EPC cells.

## Acknowledgements

Dr. Matthew C. Allender, University of Illinois – qPCR primers  
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 Dr. Jacques Robert, University of Rochester – FV3-GFP Virus  
<https://en.wikipedia.org/wiki/Daboia>  
[https://en.wikipedia.org/wiki/Common\\_box\\_turtle](https://en.wikipedia.org/wiki/Common_box_turtle)  
[https://en.wikipedia.org/wiki/Fathead\\_minnow](https://en.wikipedia.org/wiki/Fathead_minnow)

All of the experimental data points fall in the range of the standard curve between 1X and (1\*10^-2) X.