Int.J.Curr.Microbiol.App.Sci (2021) *10*(03): 1206-1219

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 10 Number 02 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1003.148

Comparison of Transcriptome of Human Hepatocytes Experimentally Infected with Yellow Fever Virus Genotypes

Gustavo Moraes Holanda[1,4*], Walter Félix Franco Neto[1], Jardel Fábio Lopes Ferreira[2], Francisco Canindé Ferreira de Luna[2], Murilo Tavares Amorim[1], João Pacifico Bezerra Neto[3], Samir Mansour Moraes Casseb[1], Juarez Antônio Simões Quaresma[4] and Ana Cecília Ribeiro Cruz[1]

[1]Arbovirology and Hemorrhagic Fevers Section, Evandro Chagas Institute, Highway BR-316, km 7 s.n, Levilândia, PA, Brazil, 67030-000
[2]Institute of Biological Sciences, Federal University of Pará, S. Augusto Corrêa, 01-Guamá, Belém-PA, Brazil, 66075-110
[3]Biological Sciences Center, genetics department, Federal University of Pernambuco, Recife-PE, Brazil, 50730-120
[4]Post-graduate Program in Amazon Parasitic Biology, State of Pará University, Tv. Angustura, 2219-Pedreira, Belém-PA, Brazil, 66087-310

**Corresponding author*

Keywords

Immune response, Transcriptome, Viral infection, Yellow fever

Article Info

Accepted:
10 February 2021
Available Online:
10 March 2021

A B S T R A C T

Analysis and comparison of the transcriptome of human hepatocytes infected with the Yellow Fever virus to verify the transcriptional profile of both genotypes, in response to viral infection. The sample used corresponds to strains of YFV code H622205, from the state of Goiás-GO (Brazil), a fatal human case isolated from the patient's blood and serum. The techniques of cell cultures, extraction of viral RNA, sequencing and analysis of transcriptome, as well as the pre-processing of both the RNA and transcriptome libraries, mapping and abundance of transcripts and statistical analyzes were all performed in the Arbovirology Section of the Institute Evandro Chagas. According to the viral titration, there is a greater replication of Genotype II compared to Genotype I. In both infections, we noticed that the moment of the lowest viral load is 24 hpi and the highest viral load is 96 hpi. Regarding the VFA genotype II infecting HepG2, the same tests were carried out, but the significance of the statistical test for differential expression was less than that found in genotype II.A statistical test of formation of "HeatMap" was also performed by the RSEN software**.** The present study emphasize that this work has great value in clarifying differences in the infection of genotype I and II of YFV in human hepatocytes, in addition to being a milestone, for presenting the first transcriptome of both genotypes of YFV performed in hepatocytes.

Introduction

Yellow Fever (YF) is an infectious disease transmitted by mosquitoes of the *Culicidae* family caused by the yellow fever virus (YFV). This virus is endemic in tropical regions of Africa and South America, where, according to the World Health Organization (WHO), about 200 thousand infections occur each year, and 30 thousand result in deaths (1). Yellow fever is a zoonosis that affects primary non-human primates, humans are infected when they enter regions where the vector is present, usually forests. Its transmission to humans is due to mosquito bites of the genus *Haemagogus* (primary) and *Sabethes* (secondary) in forest areas, the two main vectors are *Haemagogus janthinomys* and *Haemagogus leucocelaenus* (2). In addition to these vectors, the species *Aedes aegypti* can also spread the virus in urban environments among man-mosquitoes, without the need for amplifying hosts (3). Thus, the cycle continues until the susceptible are exhausted or mass vaccination is carried out with the 17D vaccine strain, one of the safest and most effective live attenuated virus vaccines, blocking transmission.

The YFV penetrates the skin through the mosquito bite and is initially replicated in the regional lymph nodes. It then spreads through the bloodstream to other organs, such as the liver, kidneys, bone marrow, nervous system, heart, pancreas, spleen and lymph nodes. In these organs, where viral replication, lesions occur, being more evident in the liver, with necrosis, cell apoptosis, macro and micro steatosis and central inflamed regions (QUARESMA *et al.,* 2006; QUARESMA *et al.,* 2005). To analyze viral isolation, blood or serum is collected from patients and collected within the fourth day of the disease. These can be inoculated in animal cell cultures, such as VERO and BHK-21 cells, and in mosquito cell cultures, such as TRA-284, C6 / 36 and AP-61, in addition to newborn mice or mosquitoes. Its identification can be performed through serological tests, such as neutralization (TN), inhibition of hemagglutination (HI), complement fixation (FC), indirect immunofluorescence (IF) and immunoenzymatic assay (EIA)(6).

The immune response caused by most flaviviruses occurs after inoculation by the mosquito bite, the first round of viral replication appearing in dendritic cells and then migrating to the lymph nodes, where the antigen is presented to CD4+ T lymphocytes, viruses replicate and then the infection spreads. At this point it is known that there are substances in the mosquitoes saliva that negatively regulate the production of IFN-γ and positively IL-4 and IL-10 (7–9). At the beginning of the YFV infection, IgM antibodies appear until the second week of the disease, followed by a rapid decline over a few months. Neutralizing antibodies emerge quickly after the virus enters until the end of viremia, persisting for many years. It is also known that infection by YFV can cause cross-protection with other flaviviruses (10,11). It is also known of the great importance of IFN types I and II in inhibiting infection by flavivirus. Type I IFN (α and β) blocks infection, negatively regulating the translation and replication of the viral RNA. Type II IFN (IFN-γ) inhibits replication by generating inflammatory and antiviral molecules, such as nitric oxide (12–14).

Viruses have evolved mechanisms that attenuate IFN production that already being demonstrated in the Hepatitis C virus (HCV), blocking the transcriptional response of IFN-α(15). The complement system also inhibits viral growth from various mechanisms, such as lysing the envelope of viral particles (C5-C9), generating pro-inflammatory peptides (C3a and C5a), promoting viral elimination after opsonization and facilitating the

presentation of antigens by the C3 molecule (16–20). YFV infection can cause damage to the liver, heart, kidneys, central nervous system and especially the liver. Quaresma *et al.*, (2005) describes the histopathological characterization of the liver showing necrosis, steatosis and apoptosis of liver cells. The presence of central regions with inflammatory infiltrate is also described, with CD4 + and CD + 8 T lymphocytes in place that bind to the FasL ligand, inducing cell apoptosis (QUARESMA *et al.*, 2005). Other apoptosis cells are CD45RO, CD20, S100, CD68 and CD65 (21).

Despite the good description of the immune response to YFV to date, there is no characterization of post transcriptional variations of cellular RNAs in an infection. RNA processing is known to be one of the main factors involved in differentiation and complex cellular functionality (22). A study developed with the Dengue virus described several post-transcriptional modifications and isoforms of genes after infection by the virus, indicating the differentiation in the characterization of the cellular response (23). An example of a gene that is regulated by the Dengue virus is XBP1, which expresses a transcription factor regulating MHC class II genes, which is extremely important in cellular immunology (24).

Transcriptome studies were carried out on *Aedes aegypti* mosquitoes infected with the Dengue, Yellow Fever and West Nile viruses. It was verified around 203 genes that had a regulation increased up to 5 times and around 202 genes that had a regulation reduced up to 10 times with functions transport, metabolic processes, peptidase activity, sexual determination and ion bond proteins. Among these genes, 20 genes showed similar regulation in infection by the three viruses (25,26). In transcriptome it was also found that some important genes in the production of cytokines can be regulated positively or negatively in a viral infection. As an example the Tick-Borne Flavivirus, which in an analysis in renal cells, it was seen that the oncogenes AKT2 and ERBB2, TNF-α and CXCL10 are up-regulated, whereas the Type I Interferon genes were down-regulated (27).

Yellow fever is a public health problem, however little is known about how viruses can influence gene expression in infected cells, often hampering the immune response, such as decreasing cytokines and preventing apoptosis. In some other cases, the reverse may occur, where genes essential for cell metabolism and viral defense are activated. Because of this, we propose an analysis and comparison of the transcriptome of human hepatocytes infected with the Yellow Fever virus to verify the transcriptional profile that occurs, of both genotypes, in response to viral infection.

Materials and Methods

This work did not use animals, humans or the environment as a direct object, so it is not necessary to be evaluated by the ethics committee. Only viral samples previously collected and isolated from cell cultures belonging to the Evandro Chagas Institute, Section of Arbovirology and Hemorrhagic Fevers (SAARB / IEC) were used.

The sample used corresponds to strains of YFV code H622205, from the state of Goiás-GO (Brazil), a fatal human case isolated from the blood and serum of the patient. YFV positivity has been previously tested by immunofluorescence.

Cell culture

Vero cell culture was performed using 199 medium with the addition of 2.2 g / L of sodium bicarbonate, penicillin (100 U / mL)

and streptomycin (100 µg / mL). For cell growth, fetal bovine serum, 5%, SBF, and maintenance serum, 2%, were used. Viral strains have been used to infect up to two passages in Vero. For passages and viral stock the viral inoculum was used in a multiplicity of infection (moi) of 1.

After 4-6 days post-inoculation the supernatants were centrifuged at 8,000 RPM, for 20 minutes at 4 ° C and separated for viral titration.

HepG2 cell cultures were used because they are considered excellent cultures and widely used for experimental studies with flavivirus, including studies on secretome(28), proteomics (29), internalization and viral propagation by specific serotypes (30) and expression of genes involved in innate immunity (31), miRNAs (32), among others.

HepG2 cultures were carried out in Dulbecco's modified Eagle medium (DMEM), plus 1.5 g / L sodium bicarbonate, Hepes (10 mM), L-glutamine (2 mM), pH 7, two. The cultivation subculture was carried out when the monolayer confluence is approximately 70%. The cells were incubated in growth medium for up to 72 hours before inoculation.

For inoculation, the growth medium was removed, and the monolayers washed twice with PBS, and inoculated with MOI 1, for this the viral samples were diluted in a medium similar to that of maintenance, but with 2% SBF, standardizing a volume. The bottles were incubated for 1 hour in a 37 ° C oven at 5% CO2 and regularly shaken. After viral adsorption, the viral inoculums were removed and the monolayers washed up to twice with PBS, after which 8 ml of maintenance medium was added, and finally incubated in a 37 ° C at 5% CO2 for up to 96 hours. Every 24 hours post infection (hpi), a bottle was removed for the extraction of mRNAs and viral titration.

RNA extraction

The mRNAs were extracted with the Maxwell® 16 Cell LEV RNA Purification Kit (Promega® / USA) from the suspension of infected cultures, following the manufacturer's recommendations every 24 hpi, up to 96 hpi. The viral RNA was extracted by the Maxwell® 16 Viral Total Nucleic Acid Purification Kit (Promega® / USA), following the manufacturer's protocol. Quantification of viral RNA was previously performed by (33).

Transcriptome sequencing and analysis

The long RNA libraries were built from 300 ng of total RNA extracted from infected cells and the negative control (Mock) was enriched for poly-A using the Dynabeads® mRNA DIRECT ™ Purification Kit (Ambion, USA) according to manufacturer's recommendations, and ribosomal RNA (rRNA) depletion was performed using the TruSeq Stranded Total RNA kit (Illumina) according to the manufacturer's standard protocol.

The sequencing was done on the NEXTSEQ 550 platform (Illumina), sequencing the 100 nt clones in the sense and antisense direction (2 x 100 nt). The sequencing results were made available in Fastq format and analyzed in the Galaxy online system (https://usegalaxy.org).

Pre-processing of small RNA libraries

The libraries were submitted to a quality filter using the FastQC script (34), available in the Galaxy package (https://usegalaxy.org). The parameters -q 20 and -p 60 were used, which define the minimum quality and the percentage of the total number of bases that have the minimum quality stipulated, respectively. The sequences that were passed through the quality filters were subjected to the removal of adapters using the Trimmomatic software (35). Sequences less

than 15 nt were discarded after removing the adapters.

Pre-processing of transcriptome libraries

In the case of long RNA libraries, the raw sequences are being subjected to a quality filter and removal of the adapters using the Trimmomatic software (35) with the LEADING parameters: 3 TRAILING: 3 SLIDINGWINDOW: 4: 15 MINLEN: 36, which indicate the removal of 3 low quality bases at the 5 'and 3' ends, the removal of portions in the sequence that are less than 15, and the removal of sequences that after the quality filters are less than 36.

Mapping and abundance of transcripts

The mapping of the transcriptome libraries against the reference genome was performed using the protocol described by Trapnell *et al.,* (2012) (36) with adaptations for faster software, for this, the HISAT2 package version 2.1.0 (37).

Using the HISAT2 output files, the relative expression of the genes annotated in the genome was estimated using the RSEM package (38), to performe a Timeframe. For normalization between libraries, the quartile and blind dispersion method were selected as a parameter. The quantification of gene expression is given as the frequency of fragments mapped per kilobase of the gene per million sequences mapped in the reference (FPKM - Fragments Per Kilobase of exon per Million reads mapped), corrected according to the maximum probability of the mapping happening in the gene locus to the alternative loci.

The file Homo_sapiens.GRCh38.94.gtf was used as the reference genome, available on the NCBI online platform (https://www.ncbi.nlm.nih.gov/).

Enrichment analysis

From the normalized gene expression between the libraries, the process enrichment analysis was performed with the EdgeR(39) on the R project platform. The values were normalized based on the size of the set of genes contained in each process and the rate of false positives for each process is determined. At the end of the process, graphics representing the differential expression between the days of infection and the Mock were generated for comparison.

Statistical analysis

Statistical analyzes were performed using the PrismGraph 6.0 software (GraphPad Prism® INC, EUA). The titration results, including the viremic curve, were performed by Holanda *et al.,* (2017) (33).

Results and Discussion

Viral titration

According to the viral titration, there is a greater replication of Genotype II compared to Genotype I. In both infections, we noticed that the moment of the lowest viral load is 24 hpi and the highest viral load is 96 hpi (Figure 1) (33).

Yellow Fever Genotype I

Differential expression

After using Trimmomatic and aligning with the HISAT2 (https://usegalaxy.org/), differential expression was performed between the infected samples and the MOCK (negative control) by EdgeR with a value dispersion up to 0.4, we obtained for the samples 24 hpi X MOCK, 48 hpi X MOCK, 72 hpi X MOCK and 96 h X MOCK the results shown in figure 2.

According to the results, it is noticed that there are several genes significantly expressed (red dots) and some more rarely expressed (red dots closer to the center) (Figure 2).

In addition to these analyzes, a statistical test of Heat Map formation was performed by the RSEN software, presenting the following results (Figure 3).

Yellow Fever Genotype II

Regarding the VFA genotype II infecting HepG2, the same tests were carried out, but the significance of the statistical test for differential expression was less than that found in genotype II. As shown in figure 4.

A statistical test of formation of "HeatMap" was also performed by the RSEN software, presenting the following results (Figure 5).

Related to genotype I, it is important to point out that it was possible to observe a differential change between the samples and the control, with a greater number of changes occurring at 24 hpi and 48 hpi (shown by the red dots - Figure 2), this was due to the the fact that 24 hpi there are about 350 targets (genes) (Figure 2 B) more expressed in relation to other hours and at 48 hpi there are about 228 targets (Figure 2 B) more than in the 72 hpi and 96 hpi periods.

If we relate this information to the virus replication period (shown in Figure 1), it is possible to understand that these targets must be present positively in the viral replication process, facilitating the replication of the virus in the infected cells or negatively making a cellular attempt to avoid the replication process.

As shown in figure 3, the colors in the "gradient" to the purple color are related to isoforms where it is possible to observe a greater number of them in relation to another day of infection. If we focus our attention on the second column and the line related to 24 hpi, it presents the greatest number of changes in relation to the other days with MOCK. In addition, if we analyze the 24 hpi line, this period is really the one that has the greatest difference in relation to other days, as it is clear that the quadrants related to the comparison of isoforms are tending to purple.

Fig.1 Viral titration performed by RT-qPCR in experimentally infected HepG2 cells, from 24 to 96 hours post-infection. Samples were normalized in relation to MOCK (negative control). p <0.0001 (33)

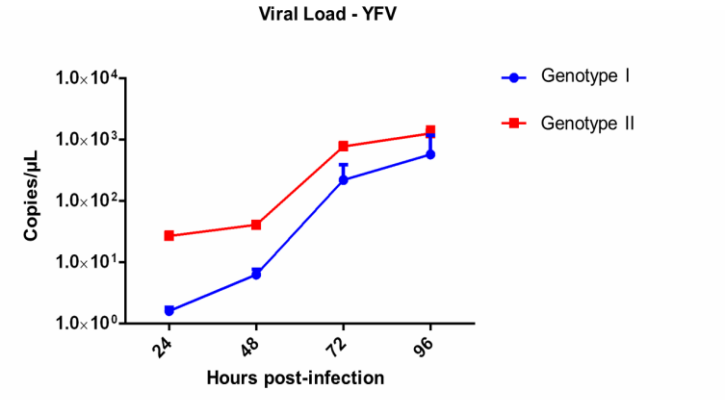


Fig.2 Differential expression between the sample of cells infected by the YFV genotype I A. 24 hpi X MOCK, B. 48 hpi X MOCK, C. 72 hpi X MOCK and D. 96 hpi X MOCK. The dots demonstrate the genes that were found in the reads. The red dots represent the genes significantly expressed. log Counts: number of reads counted in log p <0.05

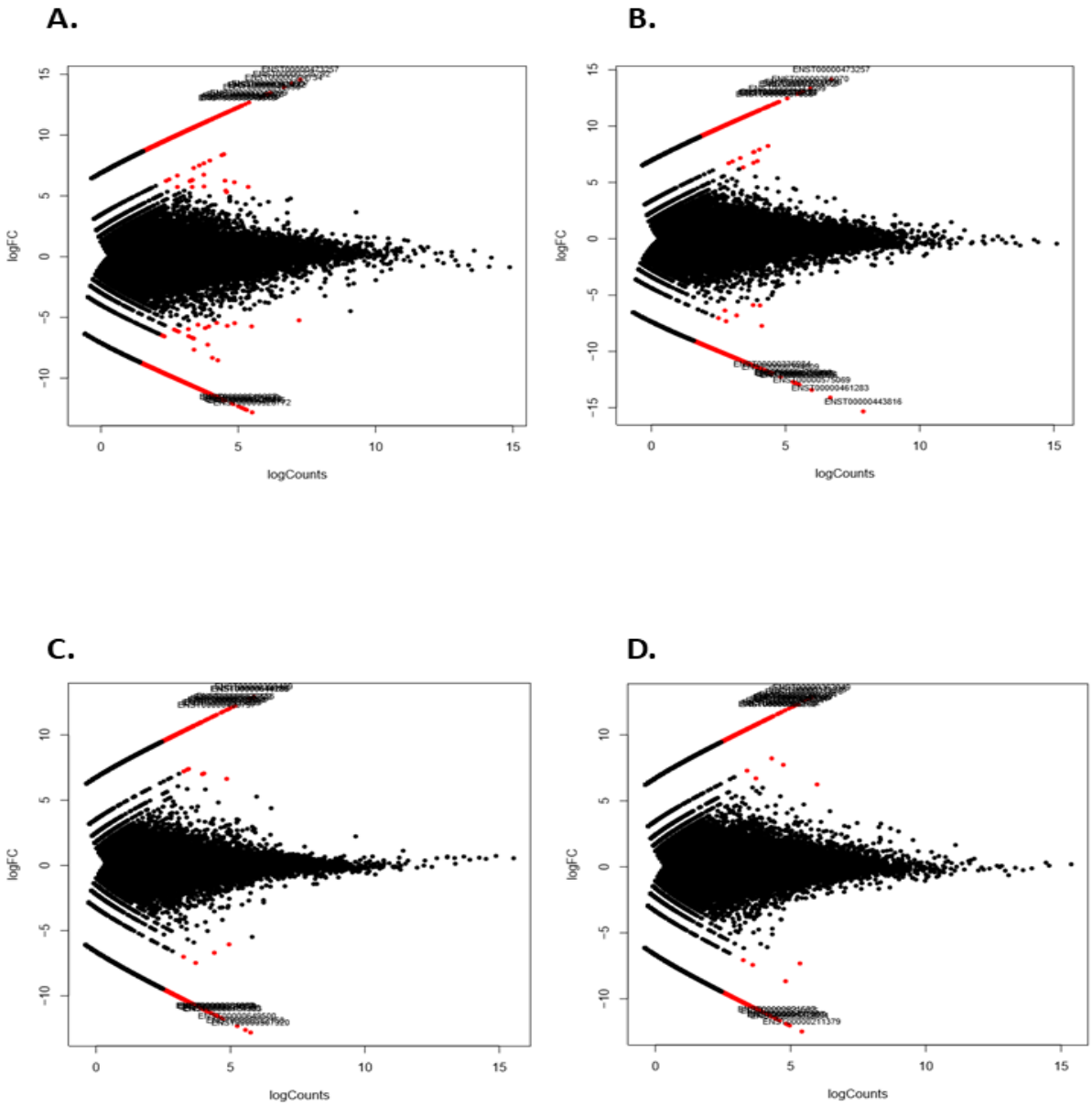


Fig.3 HeatMap showing the significance values for each sample (24, 48, 72 and 96hpi) compared to MOCK and among them of HepG2 cells infected with the YFV genotype I.

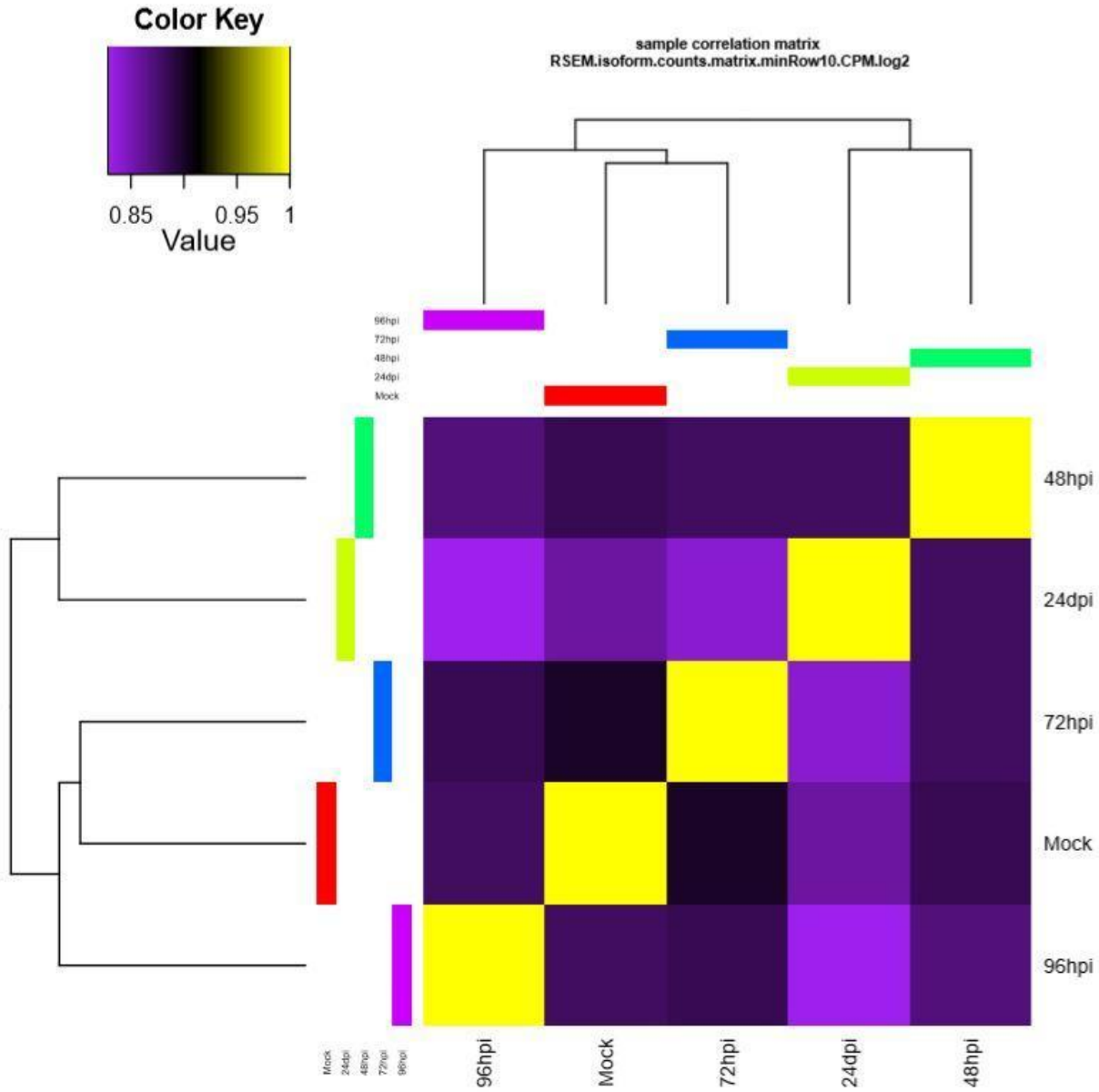


Fig.4 Differential expression between the sample of cells infected with YFV genotype II. A. 24 hpi X MOCK, B. 48 hpi X MOCK, C. 72 hpi X MOCK and D. 96 hpi X MOCK. The dots demonstrate the genes that were found in the reads. The red dots represent the genes significantly expressed. logCounts: number of reads counted in log p <0.05

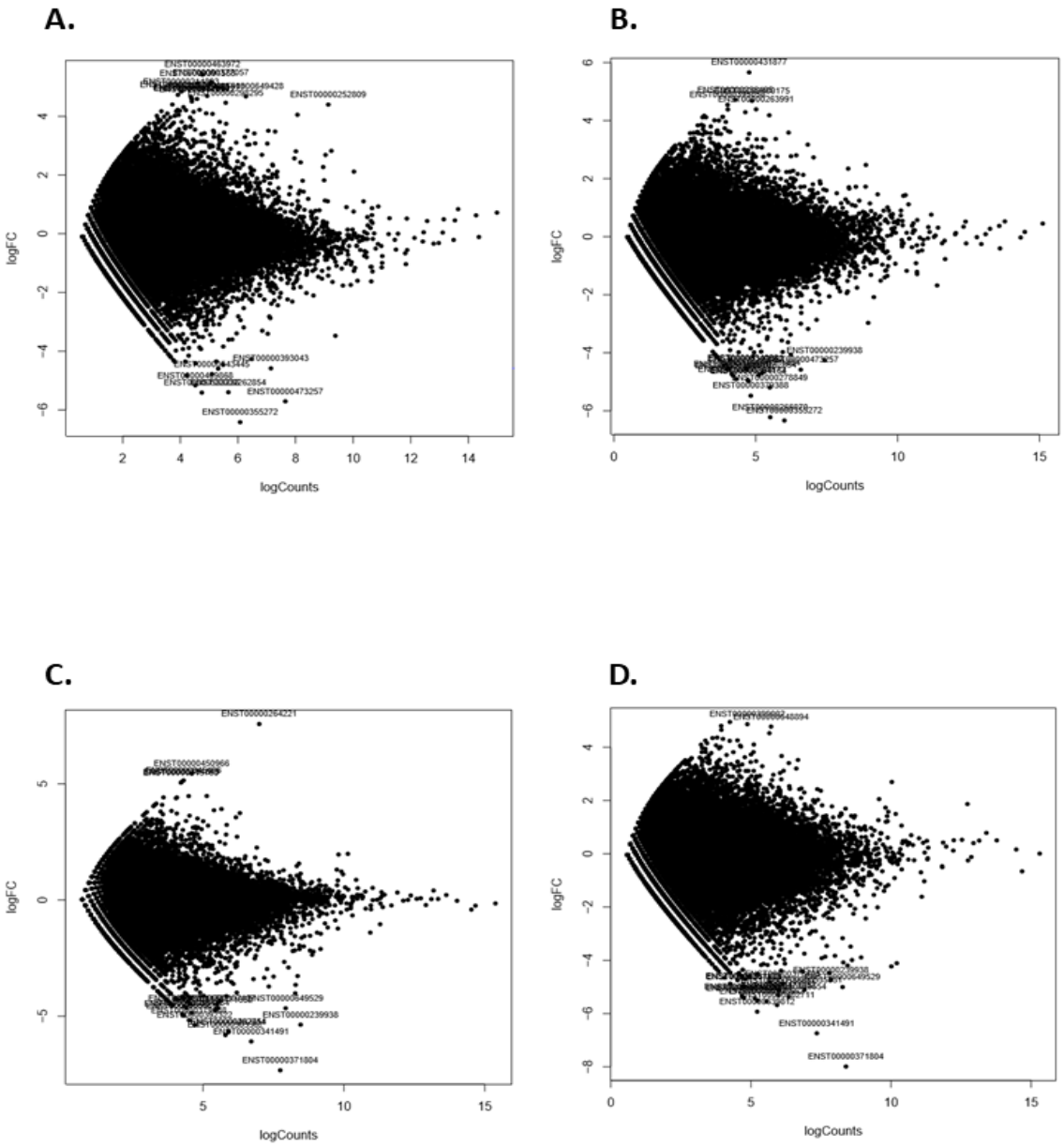
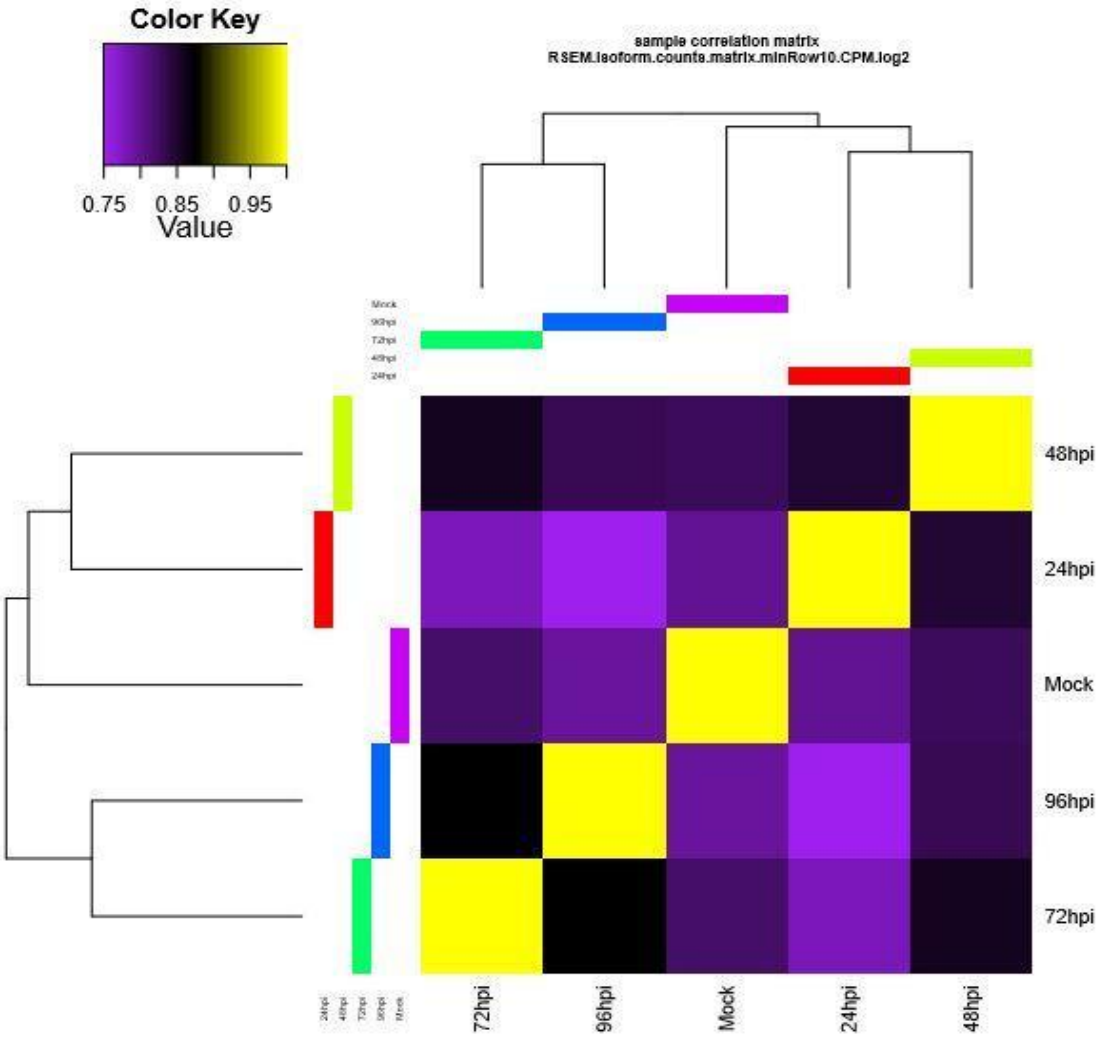


Fig.5 Heat Map showing the significance values for each sample (24, 48, 72 and 96 hpi) compared to the Mock and among them of HepG2 cells infected with the YFV genotype II.



According to the results, it is noticed that there are few genes significantly expressed (there are no red dots) in genotype II. From the most distant and closest genes to the center, those that have a proven relationship with viral infections and others with cell maintenance will be chosen for further analysis for the publication.

It is noticeable that the pattern of isoforms linked to the transcripts between genotype I and genotype II are similar, where at 24 hpi it is possible to recognize a greater number of significant isoforms in relation to MOCK and other periods of infection.

From the data presented, it was seen that there is greater differential expression of genes in cells infected by the genotype.

Few studies have carried out the transcriptome of cells infected by arboviruses, mainly in human cells, thus representing little information for comparison with other cells and other arboviruses.

Other authors focus on the relationship of gene expression in mosquito cells, which have immunological differentiation compared to human cells (26). Studies focused on vaccine strains (17D) are also found, but it is known that it induces an immune response similar to pathogenic viruses (40). But of great importance for the development and understanding of vaccines.

A direct analysis of infections in monkeys of the *Macaca mulata* species, induction of pathways associated with apoptosis, similar to that already presented in human cells, deregulation of innate immune response genes, inhibition of lymphocytes, interruption of ion binding (zinc) (33,41–43).

We emphasize that this work has great value in clarifying differences in the infection of genotype I and II of YFV in human hepatocytes, in addition to being a milestone, for presenting the first transcriptome of both genotypes of YFV performed in hepatocytes. In the future, gene by gene will be analyzed for a publication where it will be incorporated into the genotypic differential analysis and the pattern of immune response by mRNA expression.

References

1. Akbari OS, Antoshechkin I, Amrhein H, Williams B, Diloreto R, Sandler J, *et al.*, The developmental transcriptome of the mosquito *Aedes aegypti*, an invasive species and major arbovirus vector. G3 (Bethesda) [Internet]. 2013;3(9):1493–509. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3755910&tool=pmcentrez&rendertype=abstract
2. Anderson JF, Rahal JJ. Efficacy of interferon alpha-2b and ribavirin against West Nile virus in vitro. Emerg Infect Dis. 2002;8(1):107–8.
3. Andrews S. FastQC A Quality Control tool for High Throughput Sequence Data. FastQC A Quality Control tool for High Throughput Sequence Data. 2010. http://www.bioinformatics.babraham.ac.uk/projects/.
4. Blencowe BJ. Alternative Splicing: New Insights from Global Analyses. Cell. 2006; 126(1):37–47.
5. Bolger AM, Lohse M, Usadel B. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics. 2014; 30(15): 2114–20.
6. Christophers R. Aedes aegypti, the yellow fever mosquito. Cambridge Univ Press. 1960;1.
7. Colpitts TM, Cox J, Vanlandingham DL, Feitosa FM, Cheng G, Kurscheid S, *et al.*, Alterations in the aedes aegypti transcriptome during infection with west nile, dengue and yellow fever viruses. PLoS Pathog. 2011;7(9).
8. Conceição TM, El-Bacha T, Villas-Bôas CSA, Coello G, Ramírez J, Montero-Lomeli M, *et al.*, Gene expression analysis during dengue virus infection in HepG2 cells reveals virus control of innate immune response. J Infect. 2010;60(1):65–75.
9. Da Costa XJ, Brockman M a, Alicot E, Ma M, Fischer MB, Zhou X, *et al.*, Humoral response to herpes simplex virus is complement-dependent. Proc Natl Acad Sci U S A [Internet]. 1999; 96: 12708–12. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=23060&tool=pmcentrez&rendertype=abstract
10. de Sousa JR, Azevedo RSS, Martins Filho AJ, Araujo MTF, Moutinho ERC, Baldez Vasconcelos BC, *et al.*, Correlation between Apoptosis and in Situ Immune Response in Fatal Cases of Microcephaly Caused by Zika Virus. Am J Pathol [Internet]. 2018;188(11):2644–52. Available from:

https://doi.org/10.1016/j.ajpath.2018.07.009

11. Diamond MS, Harris E. Interferon inhibits dengue virus infection by preventing translation of viral RNA through a PKR-independent mechanism. Virology [Internet]. 2001;289(2):297–311. Available from: http://www.sciencedirect.com/science/article/pii/S0042682201911146
12. Dunkelberger JR, Song W. Complement and its role in innate and adaptive immune responses. Cell Res [Internet]. 2010;20(1):34–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20010915%5Cnhttp://dx.doi.org/10.1038/cr.2009.139
13. Engelmann F, Josset L, Girke T, Park B, Barron A, Dewane J, *et al.*, Pathophysiologic and Transcriptomic Analyses of Viscerotropic Yellow Fever in a Rhesus Macaque Model. PLoS Negl Trop Dis. 2014;8(11).
14. Heim MH, Moradpour D, Blum HE. Expression of hepatitis C virus proteins inhibits signal transduction through the Jak-STAT pathway. J Virol. 1999;73(0022-538X):8469–75.
15. Higa LM, Caruso MB, Canellas F, Soares MR, Oliveira-Carvalho AL, Chapeaurouge DA, *et al.*, Secretome of HepG2 cells infected with dengue virus: Implications for pathogenesis. Biochim Biophys Acta - Proteins Proteomics [Internet]. 2008;1784(11):1607–16. Available from: http://dx.doi.org/10.1016/j.bbapap.2008.06.015
16. Ho L-J, Wang J-J, Shaio M-F, Kao C-L, Chang D-M, Han S-W, *et al.*, Infection of Human Dendritic Cells by Dengue Virus Causes Cell Maturation and Cytokine Production. J Immunol. 2001;166(3):1499–506.
17. Holanda GM, Casseb SMM, Quaresma JAS, Vasconcelos PFC, Cruz ACR. Yellow fever virus modulates cytokine mRNA expression and induces activation of caspase 3/7 in the human hepatocarcinoma cell line HepG2. Arch Virol. 2019;164(4):1187–92.
18. Holanda GM, Mansour S, Casseb M, Fabiane K, Mello L, Fernando P, *et al.*, Yellow Fever Virus Modulates the Expression of Key Proteins Related to the microRNA Pathway in the Human Hepatocarcinoma Cell Line HepG2. Viral Immunol [Internet]. 2017; 30(5): 1–6. Available from: http://online.liebertpub.com/doi/pdf/10.1089/vim.2016.0149
19. Hou J, Wang S, Jia M, Li D, Liu Y, Li Z, *et al.*, A Systems Vaccinology Approach Reveals Temporal Transcriptomic Changes of Immune Responses to the Yellow Fever 17D Vaccine. J Immunol. 2017;199(4):1476–89.
20. Kim D, Langmead B, Salzberg SL. HISAT: A fast spliced aligner with low memory requirements. Nat Methods [Internet]. 2015;12(4):357–60. Available from: http://dx.doi.org/10.1038/nmeth.3317
21. Kopf M, Abel B, Gallimore A, Carroll M, Bachmann MF. Complement component C3 promotes T-cell priming and lung migration to control acute influenza virus infection. Nat Med. 2002;8(4):373–8.
22. Li B, Dewey CN. RSEM: Accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics. 2011;12.
23. Lin YL, Huang YL, Ma SH, Yeh CT, Chiou SY, Chen LK, *et al.*, Inhibition of Japanese encephalitis virus infection by nitric oxide: antiviral effect of nitric oxide on RNA virus replication. - PubMed - NCBI. J Virol [Internet]. 1997;71(7):5227–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9188590
24. Mlera L, Lam J, Offerdahl DK, Martens

C, Sturdevant D, Turner C V, *et al.*, Transcriptome Analysis Reveals a Signature Profile for Tick-Borne Flavivirus Persistence in HEK 293T Cells. 2016;7(3):1–11.
25. Monath TP, Vasconcelos PFC. Yellow fever. J Clin Virol [Internet]. 2015;64:160–73. Available from: http://dx.doi.org/10.1016/j.jcv.2014.08.030
26. Monath TP. Yellow fever: An update. Lancet Infect Dis. 2001;1(1):11–20.
27. Ochsenbein a F, Pinschewer DD, Odermatt B, Carroll MC, Hengartner H, Zinkernagel RM. Protective T cell-independent antiviral antibody responses are dependent on complement. J Exp Med. 1999;190(8):1165–74.
28. Ochsenbein AF, Zinkernagel RM. Natural antibodies and complement link innate and acquired immunity. Immunol Today. 2000;21(12):624–30.
29. Pattanakitsakul S, Rungrojcharoenkit K, Kanlaya R, Sinchaikul S, Noisakran S, Chen S-T, *et al.*, Proteomic Analysis of Host Responses in HepG2 Cells during Dengue Virus Infection research articles. J Proteome Res [Internet]. 2007; 6: 4592–600. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17979228
30. Quaresma JA, Pagliari C, Medeiros DB, Duarte MI VP. Immunity and immune response, pathology and pathologic changes: progress and challenges in the immunopathology of yellow fever. Rev Med Virol. 2013;23(5):305–18.
31. Quaresma JAS, Barros VLRS, Pagliari C, Fernandes ER, Andrade HF, Vasconcelos PFC, *et al.*, Hepatocyte lesions and cellular immune response in yellow fever infection. Trans R Soc Trop Med Hyg. 2007;101(2):161–8.
32. Quaresma JAS, Barros VLRS, Pagliari C, Fernandes ER, Guedes F, Takakura CFH, *et al.*, Revisiting the liver in human yellow fever: Virus-induced apoptosis in hepatocytes associated with TGF-β, TNF-α and NK cells activity. Virology. 2006;345(1):22–30.
33. Robertson S, Hull B, Tomori O, Bele O, LeDuc J, Esteves K. Yellow Fever: A Decade of Reemergence. JAMA. 1996;276:1157–62.
34. Robinson MD, McCarthy DJ, Smyth GK. edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2009;26(1):139–40.
35. Romanos MTV. Febre Amarela e Dengue. In: Santos NS, Romanos M teresa, Wigg MD, editors. Introdução à Virologia Humana. 2nd ed. Guanabara-Koogan; 2008. p. 399–409.
36. Sessions OM, Tan Y, Goh KC, Liu Y, Tan P, Rozen S, *et al.*, Host Cell Transcriptome Profile during Wild-Type and Attenuated Dengue Virus Infection. PLoS Negl Trop Dis. 2013;7(3):1–12.
37. Simões Quaresma JA, Souza Barros VLR, Rainero Fernandes E, Pagliari C, Takakura C, Da Costa Vasconcelos PF, *et al.*, Reconsideration of histopathology and ultrastructural aspects of the human liver in yellow fever. Acta Trop. 2005;94(2):116–27.
38. Thepparit C, Smith DR. Serotype-Specific Entry of Dengue Virus into Liver Cells : Identification of the Laminin Receptor as a Dengue Virus Serotype 1 Receptor Serotype-Specific Entry of Dengue Virus into Liver Cells : Identification of the 37-Kilodalton / 67-Kilodalton High-Aff. J Virol. 2004;78(22):12647–56.
39. Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, *et al.*, Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat Protoc. 2012;7(3):562–78.
40. Vasconcelos PF da C. Febre amarela. Rev Soc Bras Med Trop. 2003;36(2):275–93.
41. Wu SJ, Grouard-Vogel G, Sun W,

Mascola JR, Brachtel E, Putvatana R, *et al.,* Human skin Langerhans cells are targets of dengue virus infection. Nat Med [Internet]. 2000; 6(7):816–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10888933

42. Yu C-Y, Hsu Y-W, Liao C-L, Lin Y-L. Flavivirus infection activates the XBP1 pathway of the unfolded protein response to cope with endoplasmic reticulum stress. J Virol [Internet]. 2006;80(23):11868–80. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1642612&tool=pmcentrez&rendertype=abstract

43. Zeidner NS, Higgs S, Happ CM, Beaty BJ, Miller BR. Mosquito feeding modulates Th1 and Th2 cytokines in flavivirus susceptible mice: An effect mimicked by injection of sialokinins, but not demonstrated in flavivirus resistant mice. Parasite Immunol. 1999; 21(1): 35–44.

How to cite this article:

Gustavo Moraes Holanda, Walter Félix Franco Neto, Jardel Fábio Lopes Ferreira, Francisco Canindé Ferreira de Luna, Murilo Tavares Amorim, João Pacifico Bezerra Neto, Samir Mansour Moraes Casseb, Juarez Antônio Simões Quaresma and Ana Cecília Ribeiro Cruz. 2021. Comparison of Transcriptome of Human Hepatocytes Experimentally Infected with Yellow Fever Virus Genotypes. *Int.J.Curr.Microbiol.App.Sci.* 10(03): 1206-1219.
doi: https://doi.org/10.20546/ijcmas.2021.1003.148