

## Major Article

# Comparative assessment of the replication efficiency of dengue, yellow fever, and chikungunya arboviruses in some insect and mammalian cell lines

Nidya Alexandra Segura Guerrero<sup>[1]</sup> and Felio Jesús Bello<sup>[2]</sup>

[1]. Universidad Pedagógica y Tecnológica de Colombia, Faculty of Science, Laboratory of Medical and Forensic Entomology, Tunja, Colombia.  
[2]. Universidad de La Salle, Faculty of Agricultural and Livestock Sciences, Program of Veterinary Medicine, Bogotá, Colombia.

### Abstract

**Introduction:** Insect cell cultures play an essential role in understanding arboviral replication. However, the replicative efficiency of some of these viruses such as dengue (DENV), yellow fever (YFV), and chikungunya (CHIKV) in a new cellular substrate (Lulo) and in the other two recognized cell lines has not been comparatively assessed. **Methods:** Vero, C6/36, and Lulo cell lines were infected with DENV, YFV, and CHIKV. The viral progeny was quantified through plaque assays and quantitative reverse transcription-polymerase chain reaction, while for DENV2, the findings were confirmed by immunofluorescence antibody assay. **Results:** The higher DENV2 titer (from multiplicity of infection 0.001) was obtained on day four post-infection in C6/36 and on day six in Vero cells, while the Lulo cell line was almost impossible to infect under the same conditions. However, C6/36 showed the highest values of viral RNA production compared to Vero cells, while the quantification of the viral RNA in Lulo cells showed high levels of viral genomes, which had no correlation to the infectious viral particles. **Conclusions:** C6/36 was the most efficient cell line in the alpha and flavivirus production, followed by Vero cells. Thus, Lulo cells may be a useful substrate to study the mechanisms by which cells evade viral replication.

**Keywords:** Flavivirus. Alphavirus. C6/36. Vero. Lulo. Replication efficiency.

### INTRODUCTION

Dengue virus (DENV) belongs to the Flaviviridae family, while chikungunya virus (CHIKV) belongs to the Togaviridae family. DENV and CHIKV are the most common vector-borne viral diseases in humans. In addition, both viruses are distributed in tropical and subtropical regions, and both are transmitted by *Aedes aegypti* and *Aedes albopictus*<sup>1</sup>. Bhatt *et al.* (2013)<sup>2</sup> estimated that 390 million dengue infections occur each year. The dengue mortality rate varies from 1.2 to 3.5%<sup>3</sup>. In contrast, fatality rates for CHIKV infections are approximately 1 in 1,000. Nevertheless, approximately half of the patients can present signs of persistent polyarthralgia<sup>4-6</sup>.

Yellow fever virus (YFV) belongs to the *Flavivirus* genus and causes hemorrhagic fever in humans. Despite the availability of effective vaccines, it led to 300 deaths between 2015 and 2016<sup>7</sup>. This disease is primarily maintained through a sylvatic cycle between its non-human primate hosts and sylvatic mosquito vectors<sup>8,9</sup>. When epizootics of YFV occur, humans can be infected if they live in areas near the forest or when they enter the sylvatic environment<sup>9</sup>.

In nature, mosquitoes and mammals play an essential role in propagating arboviruses. With respect to the successful propagation of arboviruses in insect and mammalian cells, both types of cells can share or present unique host factors<sup>10</sup> and, at least in part, the host factors can explain similarities as well as differences in the replication efficiency of these viruses.

Vero cell line was derived from the kidney of a *Cercopithecus aethiops* adult (African green monkey)<sup>11</sup>. However, using mitochondrial DNA analysis, it was established that Vero cells come from *Chlorocebus sabaeus*<sup>12</sup>. The characteristic that makes Vero cells one of the most frequently used cell lines in virology is their high susceptibility to arbo-<sup>13,14</sup>, adeno-<sup>15</sup>, entero-<sup>16</sup>, and influenza

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**Corresponding author:** Dr. Nidya Alexandra Segura Guerrero.  
**e-mail:** nidya.segura@uptc.edu.co  
**Orcid:** 0000-0001-5810-4847  
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viruses<sup>17</sup>. This cell line has displayed different karyotypes, with chromosomes ranging from 52 to 62<sup>12</sup>. In addition, the Vero cell line has been broadly used for the development of vaccines such as those against poliomyelitis, rabies, rotavirus, smallpox, and influenza<sup>18-20</sup>.

C6/36 is a genetically homogeneous clonal cell line<sup>21</sup> derived from *Aedes albopictus* larval tissues<sup>21</sup>. C6/36 cell line is highly susceptible to different arboviruses<sup>22,23</sup>. The predominant number of chromosomes in these cells is six, with some tetraploid types<sup>21</sup>. This cell line is easy to handle in a laboratory, because it can be incubated at room temperature for up to two weeks without changing the medium cell culture. Additionally, it has a high split ratio of 1:10 and grows into a confluent monolayer in four days<sup>24</sup>.

Lulo is a cell line derived from *Lutzomyia longipalpis* neonate larvae. Although Lulo has been used and validated for studying parasites from the *Leishmania* genera<sup>25-27</sup>, it has been established that this cell line is susceptible to infection by arboviruses such as Mayaro (MAYV), Ilheus, Changuinola, phlebo-, and vesicular stomatitis viruses<sup>28</sup>. Karyological analysis has revealed the presence of haploid, aneuploid, and tetraploid cells. However, approximately 85% of the cells presented eight chromosomes<sup>28</sup>. Similarly to C6/36, this cell line is easy to handle in a laboratory and can be incubated at room temperature. Furthermore, trypsinization is not required to detach the monolayer from the substratum. Recently, two novel RNA viruses were identified from Lulo cells, causing persistent infection in these cells. The viruses belong to two separate genera, *Luteovirus* and *Alphapermutotetravirus*, and were named *Lulo virus 1* and *2*, respectively<sup>29</sup>.

Although Vero and C6/36 cell lines are routinely used to propagate different species of arboviruses, no previous studies have assessed the replicative efficiency of DENV, YFV, and CHIKV simultaneously in each of these cellular substrates. Moreover, to our knowledge, this is the first report of the susceptibility and replication efficiency of these viruses in the Lulo cell line, comparing its replication with the other two cell lines.

## METHODS

### Cells and viruses

Vero-A (ATCC CCL-81) cells were maintained in a minimal essential medium (MEM, Gibco, Belgium) supplemented with 10% fetal bovine serum (FBS; Integro, The Netherlands), 1% L-glutamine (Gibco), and 1% sodium bicarbonate (Gibco). Baby hamster kidney cells (BHK-21; ATCC CCL10) were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen) and supplemented with 10% FBS and 1 mM sodium pyruvate (Invitrogen). C6/36 (ATCC CRL-1660) cells were maintained in Grace's insect medium (Grace Life Technologies, Belgium) and supplemented with 10% FBS and 1% L-glutamine; Lulo cells were maintained in a mix 1:1 of Grace and Leibovitz media (L-15 medium, Gibco, Belgium) and supplemented with 10% FBS. Insect cell cultures were maintained at 28 °C without CO<sub>2</sub>, while the mammalian cell culture was maintained at 37 °C in an atmosphere of 5% CO<sub>2</sub> and 95% humidity.

DENV serotype 2 New Guinea C (NGC) strain was propagated in C6/36 cells. YFV-17D vaccine strain (Aventis Pasteur) was passaged in Vero cells. CHIKV Indian Ocean strain 899 was cultured in Vero cells.

### Viral infections in insect and mammalian cells

Vero, C6/36, and Lulo cells were seeded in 12.5-mL flasks (Falcon®) using growth media. The number of cells seeded for each cell line was different in order to reach 70–80% confluence during the following 24 h. The growth medium was replaced with 2 mL of assay medium supplemented with 2% instead of 10% FBS containing the appropriate virus dilution. Vero, C6/36, and Lulo monolayers were infected with DENV2 NGC (multiplicity of infection (MOI) of 0.001), YFV (MOI of 0.001), and CHIKV 899 (MOIs of 0.001 and 0.00001). In addition, Lulo cells were infected with DENV2 NGC at a MOI of 0.1 and 0.5. After 2 h of incubation at 20–22 °C, cell monolayers were washed with phosphate buffered saline (PBS) in order to remove non-adsorbed viruses, and cultures were further incubated. Flasks were monitored daily for cytopathic effect (CPE) development, and cells and supernatants were harvested in all experiments every 48 h, except for CHIKV 899 infected at the lowest MOI, where cells and supernatants were harvested at 6, 16, 28, 40, 52, 64, and 76 h post-infection (p.i.). Total and viral RNA were isolated using the NucleoSpin® RNA (Macherey-Nagel, Düren) and the NucleoSpin 96 virus kit (Macherey-Nagel), respectively.

### DENV binding and entry assay

A binding assay was carried out in order to determine whether the viral infection detected by the plaque assay and by the immunofluorescence antibody assay (IFA) in the supernatant from Lulo infected at high MOI was due to virus replication and not from carry-over. Thus,  $1 \times 10^6$  C6/36 cells and 900,000 Lulo cells were seeded in 12.5-mL flasks (Falcon®) using the growth medium. Following 24 h of incubation, the growth medium was replaced with 2 mL of assay medium at 4 °C. DENV2 NGC infections were carried out at 4 °C using an MOI of 0.05, and cells were incubated at the same temperature for 20 min. Later, monolayers were washed with PBS, cells were immediately harvested, and total RNA was isolated using the NucleoSpin® RNA (Macherey-Nagel). In order to establish the successful entry of DENV in these cell lines, the same protocol was followed, but, this time, the assay was carried out at room temperature, and the time of incubation was extended to 45 min.

### Plaque assay

Baby hamster kidney (BHK) cells were seeded in 12-well plates (IWAKI) at a density of  $5 \times 10^5$  cells/well in 10% FBS medium. Following 24 h of incubation, monolayers were washed with PBS, and cells were infected with 500 µL of serial 1:10 supernatant dilutions, previously prepared in the assay medium. After an hour of infection, monolayers were washed with PBS in order to remove non-adsorbed viruses. Each monolayer was coated with 1.5 mL of a solution containing 2% Avicel PH-101 (Sigma-Aldrich) and 2% FBS medium. For DENV, monolayers were washed with PBS after six days of incubation; for CHIKV,

monolayers were washed after five days of incubation. Finally, monolayers were fixed with 70% ethanol and stained with blue methylene in order to visualize and count the plaque-forming units (PFU).

#### **DENV2, YFV, and CHIKV quantitative reverse transcription-polymerase chain reaction (qRT-PCR)**

DENV primers and probe sequences were as follows: DENV-For 5'-TCGGAGCCGGAGTTTACAAA-3', DENV-Rev 5'-TCTTAACGTCCGCCCATGAT-3', DENV-Probe FAM-5'-ATTCCACACAATGTGGCAT-MGB-3'<sup>30</sup>; CHIKV primers and probe sequences were as follows: ChikSII 5'-CCGACTCAACCATCCTGGAT-3', ChikAsII 5'-GGCAGACGCAGTGGTACTTCCT-3' and ChikProbe 5'-FAM-TCCGACATCATCCTCCTTGCTGGC-TAMRA-3'<sup>31</sup>; and YFV primers and probe sequences were as follows: YFV-For 5'-TGGCATATTCCAGTCAACCTTCT-3', YFV-Rev 5'-GAAGCCCAAGATGGAATCAACT-3' and YFV-Probe FAM-5'-TTCCACACAATGTGGCATG-MGB-3'<sup>30</sup>. One-step, qRT-PCR was carried out, containing 1× of master mix (Eurogentec, Seraing), 900 nM of forward primer, 900 nM of reverse primer, 200 nM of probe, 0.125 U/mL of reverse transcriptase (Eurogentec), 10 to 100 ng of RNA template, and RNase free water. qRT-PCR was carried out using the ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Branchburg) under the following conditions: 30 min at 48 °C and 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Data were analyzed using the ABI PRISM 7500 SDS software (version 1.3.1; Applied Biosystems). For absolute quantification, standard curves were generated using 10-fold dilutions of template preparation of known concentrations.

#### **IFA**

BHK cells were seeded on an 8-well chamber slide (Lab-tek, II, Nune) at a density of 8,000 cells/well; 24 h later, cells were infected using 1:10 serial dilutions of DENV2 NGC supernatants obtained from infection assays in the three cell lines at a MOI of 0.001. Furthermore, supernatants obtained from Lulo infection assays at a MOI of 0.01 and 0.05 were used. The virus inoculum was removed after 1 h, cells were washed and incubated for 72 h. Cells were stained with the anti-dengue E protein antibody (Ab) clone 3H5 (Millipore) and the secondary antibody Alexa Fluor 488 (Millipore). Following 4',6-diamino-2-fenilindol (DAPI) staining, the cultures were visualized using a confocal laser-scanning microscope (Leica Microsystems).

## **RESULTS**

### **Microscopical findings during viral infections**

When C6/36 and Lulo cells were infected with DENV2 (MOI 0.001, 0.01, and 0.05), they did not show CPE signs. Nevertheless, C6/36 infected cells started to die sooner than the mock-infected C6/36 cells. During DENV2 infection, a state of "crisis" was observed in the C6/36 culture on day six p.i., during which the cells showed poor growth. However, the cell line overcame this state on day eight p.i. In contrast, because of over confluence, Lulo-infected cells and mock-infected cells started

to form cell clumps by day 10 p.i. Contrarily, Vero-infected cell behavior (MOI 0.001) was completely different, and a strong CPE was observed after day four p.i., and this cell culture started to die on day six p.i. C6/36, and Vero cells had died by day 10 p.i., and Lulo cell cultures were not in good conditions. Whereas, CPE observed during YFV infection was very similar to CPE observed when cells were infected with DENV2.

On the contrary, when the three cell cultures were infected with CHIKV, CPE was extremely strong in Vero cells, evident in C6/36 cells, but indistinct in Lulo cells (**Figure 1**).

### **Viral production of flaviviruses and alphaviruses in insect and mammalian cell**

#### **DENV2 production**

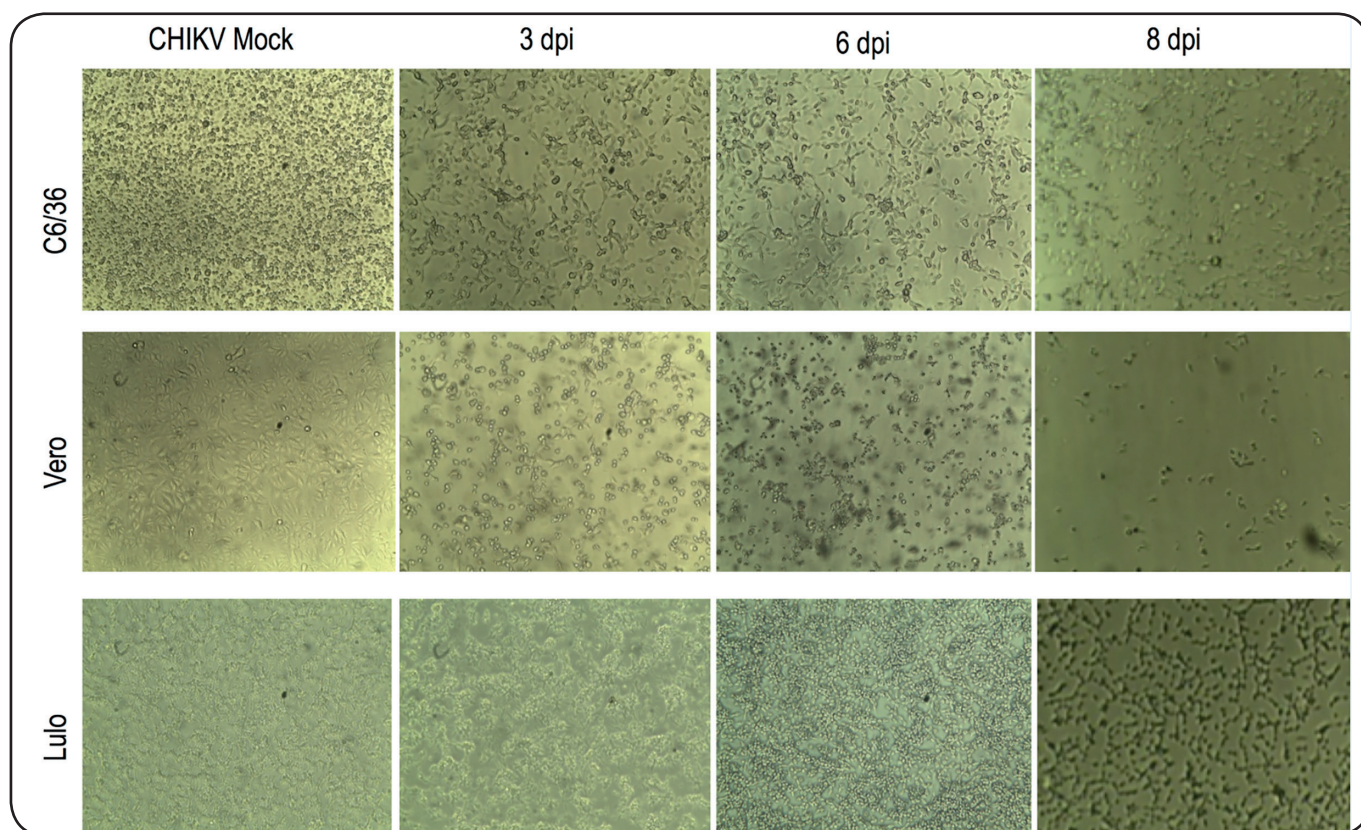
The viral progeny able to infect new cells were quantified through plaque assays using 1:10 dilutions of supernatants obtained previously. In general, during DENV2 infection (MOI 0.001), C6/36 culture was able to produce a higher number of infectious viral particles (2.8 to 4.5 Log PFU/mL) compared to the supernatant obtained from Vero cells (2.3 to 3.1 Log), and the highest DENV2 titer was obtained on day four p.i. in C6/36 (4.5 Log) and on day six in Vero cells (3.1 Log). Although the Lulo insect cell line was infected under these conditions, it showed the lowest values of DENV2 RNA production. In addition, it was not able to produce detectable infectious DENV2 by plaque assay.

These findings were confirmed by IFA, which showed that the highest DENV2 titers were produced by C6/36, followed by Vero, and that Lulo cells were not infected. Therefore, infectious DENV2 was not produced in the latter cells (data not shown).

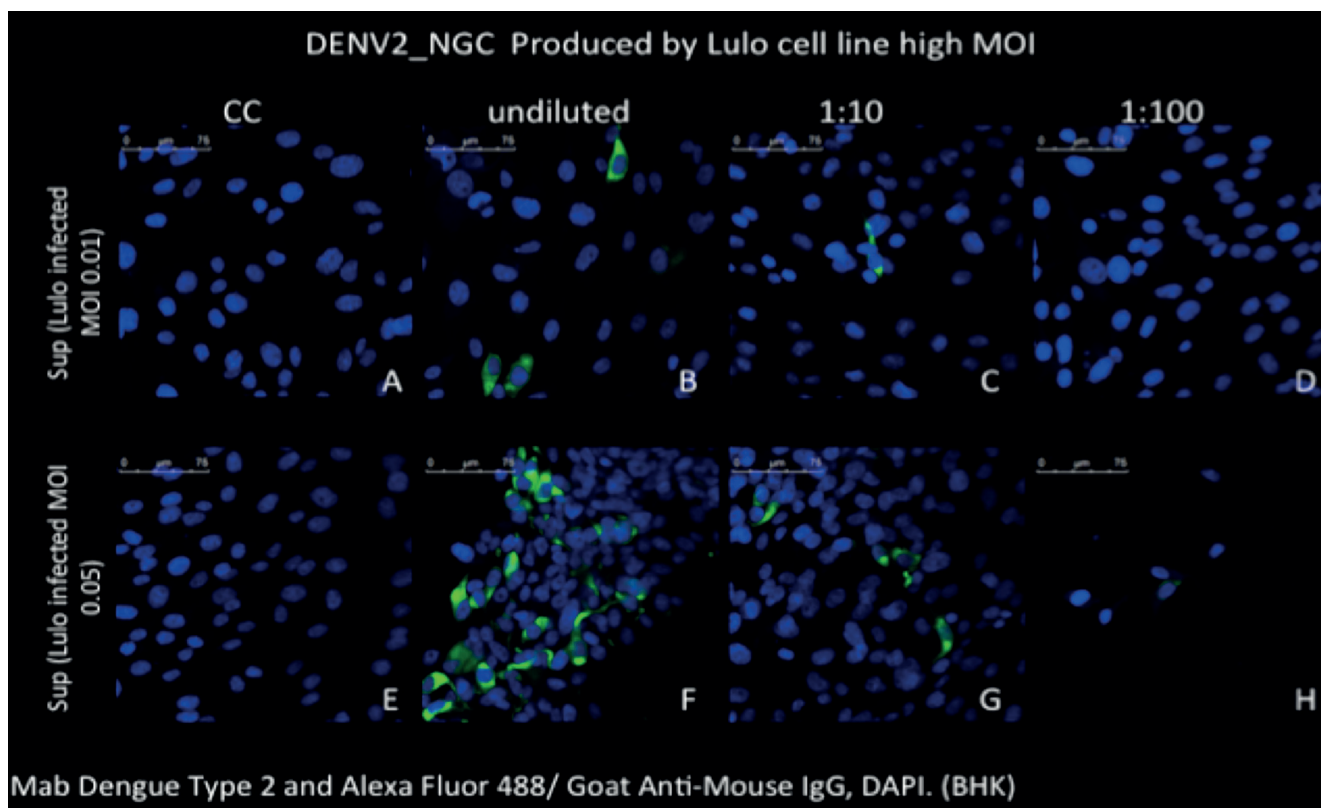
When Lulo cells were infected at high MOIs (0.01 and 0.05) with DENV2 and assessed by plaque assay, infectious DENV2 was produced as soon as day two p.i., and these viral productions showed a slow increase every day up to day 10 p.i. In addition, although there were no statistical differences, the viral production was slightly higher when cells were infected at a MOI of 0.05 (2.7 Log) rather than a MOI of 0.01 (2.9 Log).

The infectious virus titer in the supernatant from Lulo infected cell cultures was determined by IFA, which further confirmed the production of infectious virus particles (**Figure 2**). Additionally, a binding assay was carried out in order to determine whether the viral infection, detected by plaque assay and by IFA in the supernatant from Lulo cells infected at high MOI, was due to virus replication and not carry-over. Surprisingly, DENV2 was found to attach more efficiently to Lulo cells than to C6/36 cells (data not shown). Therefore, in Lulo cells, the low efficiency of viral progeny production was not due to poor attachment. In fact, DENV2 bound more efficiently to Lulo cells than C6/36 cells, although DENV2 entry was very similar between both insect cell cultures, being slightly higher in C6/36 cells.

DENV2 RNA production was assessed by qRT-PCR in infected cell supernatant at a MOI of 0.001. The viral RNA content in C6/36 and Vero cells showed high values. In the same way, the viral progeny production obtained by the plaque assay was elevated. Although viral RNA was detected in Lulo



**FIGURE 1:** Cytopathic effect at different days post infection (dpi) in insect and mammalian cells infected with CHIKV (MOI 0.001); mock-infected cells were used as uninfected cell controls (CHIKV Mock).



**FIGURE 2:** Lulo cells are able to produce infectious DENV2. BHK cells infected with DENV2 1:10 serial dilutions from Lulo cell cultures infected at MOIs of 0.01 (panels B, C, and D) and 0.05 (F, G, and H); mock-infected cells were used as uninfected cell controls (panels A and E). DENV2 E protein expression was visualized on day 3 p.i., (anti-dengue E protein antibody clone 3H5, antibody Alexa Fluor 488 (green), and DAPI staining (blue)).

supernatant cells during the assay, except on day four p.i., its levels were very low compared to the viral RNA detected in C6/36 and Vero (**Figure 3**). In addition, when Lulo supernatant (MOI 0.001) was assessed by plaque assay or by IFA, it was not able to infect BHK and Vero cells.

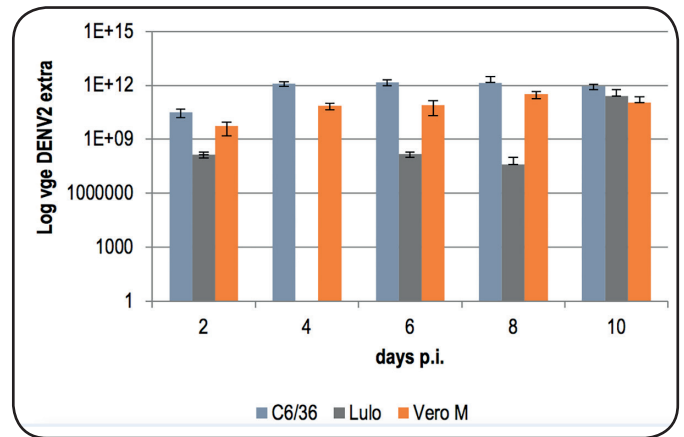
### YFV production

Intracellular and extracellular RNA from Vero, Lulo, and C6/36 infected at a MOI of 0.01 was quantified by qRT-PCR.

During the first four days of infection, the amount of released viral RNA was undetectable in the three cell cultures, and it remained undetectable during all the assays in Lulo cells. After day six p.i., the number of YFV genomes increased in C6/36 and Vero cultures, until they reached high levels (**Figure 4, panel A**). In line with the results obtained for DENV2, Lulo cell cultures showed the lowest number of viral genomes when detected by qRT-PCR. In contrast, when intracellular YFV RNA was assessed, it was detected in a relatively low amount in the three cell cultures as soon as day two p.i., but, after day four p.i., there was a high increase in the number of viral genomes produced by Vero and C6/36 but not by Lulo cells (**Figure 4, panel B**). There was no difference between the number of viral genomes (intra and extracellular) that were detected in C6/36 and Vero cells. Conversely, it is worth noting that Lulo cells were not able to produce extracellular viral RNA, despite the fact that viral RNA was detected intracellularly.

### CHIKV production

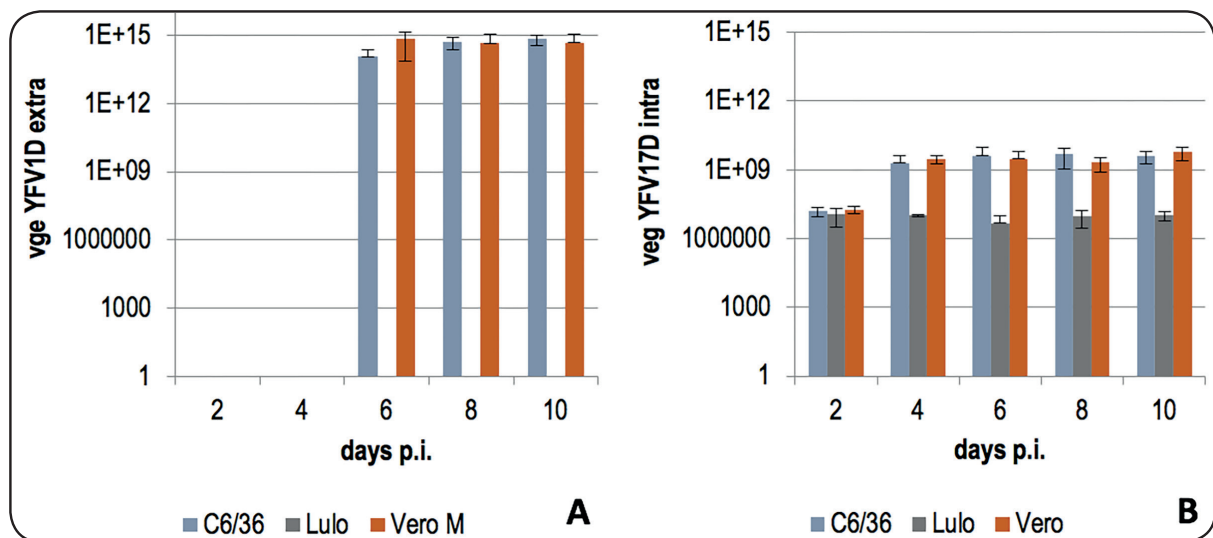
As for DENV2, when the cell lines were infected with CHIKV at an MOI of 0.001 and assessed by plaque assay, Lulo cells required a high MOI in order to produce infectious progeny (1–1.4 Log). On the contrary, C6/36 (3.1–4.5 Log) and Vero (1.7–5.6 Log) cells proved to be the most efficient cultures for viral replication, because both cell lines produced 4 logs higher infectious CHIKV compared to Lulo cultures.



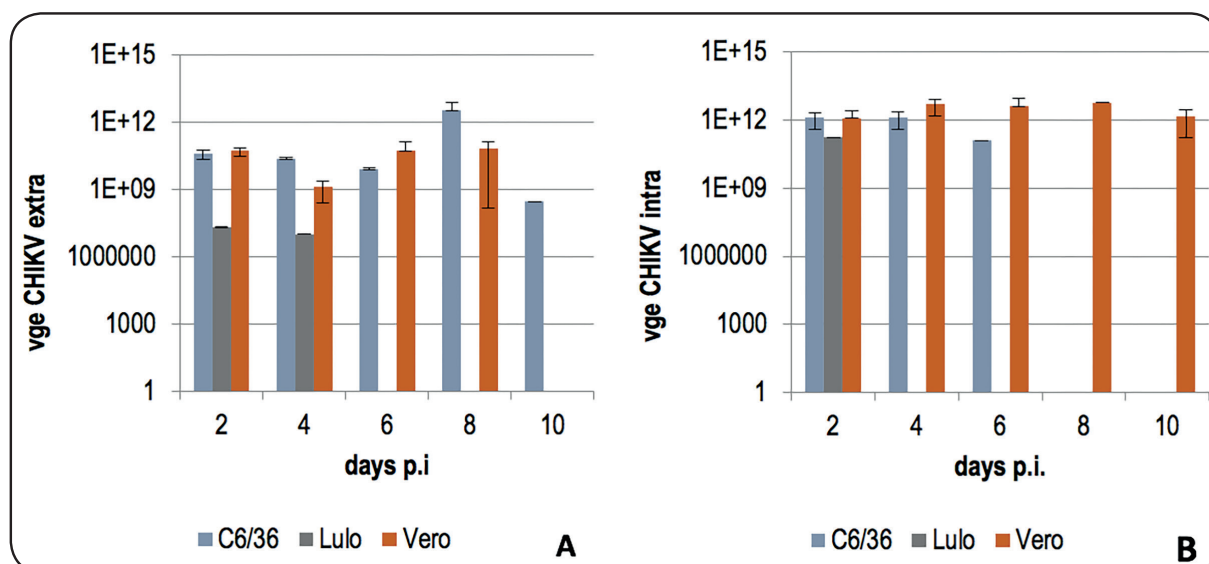
**FIGURE 3:** Release of DENV2 RNA in infected insect and mammalian cell cultures (MOI of 0.001). Production was calculated by qRT-PCR as viral genome numbers. Data are mean values of three independent experiments.

Because of the short time required for CHIKV to complete its life cycle, we decided to establish the replication at low MOI and assess the viral production in shorter periods of time. As for CHIKV, when cultures were infected at a very low MOI of 0.00001, significant differences were observed between cell lines. Lulo cells were impossible to infect under these conditions, whereas C6/36 and Vero cells showed 2.9 to 5.5 Log and 1.4 to 3.9 Log, respectively. Noticeably, viral production was more efficient in C6/36 cells than that in Vero cells.

Furthermore, CHIKV RNA production was assessed by qRT-PCR when cells were infected at high or low MOI. Extracellular viral RNA content in C6/36 and Vero cells was very similar from days two to eight. However, on day 10 p.i., viral RNA was only detectable at low levels in C6/36 cells. Viral RNA in Lulo was detectable at low levels only during the first days of infection. In addition, in agreement with the findings for DENV2, Lulo needs to be infected at high MOIs in order to be detected by



**FIGURE 4:** *In vitro* YFV replication efficiency in insect and mammalian cell cultures. Panel A: YFV released during the infection at a MOI of 0.01 in C6/36, Lulo, and Vero cells; Panel B: Intracellular YFV produced during the infection at a MOI of 0.01 in C6/36, Lulo, and Vero cells. Production was calculated by qRT-PCR as viral genome numbers. Data comprise mean values of three independent experiments.



**FIGURE 5:** *In vitro* CHIKV replication efficiency at high MOI. Panel A: CHIKV RNA released during the infection at a high MOI of 0.001 in C6/36, Lulo, and Vero cells; Panel B: Intracellular CHIKV RNA produced during the infection at a MOI of 0.001 in C6/36, Lulo, and Vero cells. Production was calculated by qRT-PCR as viral genome numbers. Data comprise mean values of three independent experiments.

qRT-PCR (**Figure 5, panel A**). The highest levels of intracellular CHIKV RNA corresponded to Vero cells on days six to ten p.i. There was no difference between C6/36 and Vero cells on days two to four. By contrast, as it was shown before, Lulo cell culture was almost impossible to infect under these conditions (**Figure 5, panel B**). Contrary to previous findings, when cell cultures were infected at a low MOI and assessed by qRT-PCR, C6/36 showed the highest intra- and extra-cellular values of viral genomes, followed by Vero cells, and Lulo cells showed a very low number of CHIKV genomes (data not shown).

## DISCUSSION

Insect and mammalian cell lines are used for different purposes such as viral propagation, study of antiviral strategies<sup>32,33</sup>, characterization of proteins<sup>34</sup> and vaccines<sup>18,19</sup>, and study of host factors<sup>10,35</sup>. However, there are several essential factors that can influence the replication efficiency of arboviruses in cell cultures such as type (serotype and strain) of virus,<sup>36,37</sup> virion binding, cell receptors<sup>38,39</sup>, endocytosis of the viral particles,<sup>40</sup> and host factors that can enable successful viral replication<sup>10</sup>, viral production, and release of new infectious viral progeny.

Although both insect and mammalian cell lines presented different characteristics when infected with flaviviruses or alphaviruses, one of the most notable characteristics was the appearance of CPE in Vero cells. On the contrary, CPE was moderate in C6/36 cells when they were infected with CHIKV and imperceptible when the cell culture was infected with DENV or YFV. In the case of the Lulo cell culture, CPE was not present when cells were infected with flaviviruses or alphaviruses. These findings are in agreement with previous reports that indicate that CPE is almost absent in insect cells<sup>41,42</sup>. In both insect and mammalian cells, this can be explained due to the fact

that DENV infection activates the unfolding protein response (UPR) in order to cope with ER stress. This is a protective mechanism that can protect cells from apoptosis, allowing viral replication<sup>43,44</sup>. When UPR is activated in DENV-infected cells, they ultimately face apoptosis because of the effect of ER stress or mitochondrion-mediated caspase pathway. This can result in changes in the mitochondrial membrane potential and the appearance of reactive oxygen species (ROS), given that mosquito but not mammalian cells increase the activities of enzymes such as glutathione S-transferase that play a role in cellular detoxification<sup>41</sup>. During CHIKV infection, it is suggested that C6/36 but not Vero cells can carry some host factors that are able to protect this cell line against apoptosis<sup>42</sup>.

In the present study, C6/36 showed the highest values of viral RNA production as well as infectious progeny production, which could be associated, at least in part, to the fact that the origin of this cell line was the natural vector of DENV and CHIKV, *Ae. albopictus*. In addition, C6/36 is a clonal cell line, which can afford to have a uniform host cell system instead of a mixed population<sup>21</sup>. Another explanation for the dissimilar response in insect and mammalian cells during arboviral infections is the exogenous interference RNA (RNAi) pathway, which is an important antiviral defense against arboviruses in the Diptera order<sup>45</sup>. When C6/36 cells are infected with DENV2, they exhibit inefficient Dicer-2 cleavage of long double strand RNA (dsRNA) that recognizes and cleaves dsRNA into siRNA in order to initiate the RNAi pathway. The ability of C6/36 to support robust arbovirus replication can be due to lack of a complete, functional RNAi pathway<sup>32</sup>. This dysfunctional pattern has been also found when C6/36 cells are infected with bunyaviruses<sup>46</sup>, other flaviviruses, and alphaviruses<sup>47</sup>.

Despite the strong CPE observed during the arboviral infection in Vero cells, the cell culture showed efficient

replication of flaviviruses and alphaviruses. This finding has been previously reported, and it might have been strongly influenced by the inability of this cell line to produce IFN type 1 and antiviral factors, known as IFN-stimulated genes<sup>48,49</sup>, which allowed for extreme accommodation of Vero to viral infections.

In the case of the viral production by Lulo cells, there were surprising findings. It was observed that when Lulo cell cultures were infected at high MOI, the cell culture was able to produce infectious viral particles. When DENV binding and entry was compared to Lulo and C6/36 cells, only slight differences were found, suggesting that Lulo had an appropriate cell receptor and that the inefficient arboviral replication could be associated with downstream events or lack of proper host factors required for efficient viral production. In addition, the quantification of the viral RNA in Lulo showed high levels of viral genomes, which had no correlation to the infectious viral particles (plaque assay), opening the possibility of the production of defective viral particles. On the other hand, although *Lu. longipalpis* is not a natural vector of flaviviruses and alphaviruses, the cell line derived from this species has been reported to be susceptible to MAYV (alphavirus)<sup>28</sup>, showing that related viruses cannot necessarily replicate efficiently in the same cell culture<sup>36,37</sup>.

In conclusion, C6/36 was the most efficient cell line in the flavivirus and alphavirus production, followed by Vero cells. However, it is important to consider their characteristics for virological studies, as both cell lines do not represent an accurate model for molecular interactions, in the case of C6/36, because of lack of a functional RNAi pathway<sup>32</sup>, and, in the case of Vero, because of the inability to produce IFN type 1<sup>49</sup>. Despite this, C6/36 could be considered as an alternative to culture arboviruses such as DENV, CHIKV, and YFV, because this cell line exhibited higher or at least equal values of both viral RNA production and infectious viral progeny than those shown by Vero. In addition, C6/36 is able to remain alive for longer periods under infection conditions than Vero cells, and sometimes it is necessary to propagate arboviruses up to 2 weeks; however, Vero cell culture is not capable of being maintained for this period of time without being passaged.

Lulo cell line was almost unable to produce high levels of infectious viral progeny, showing that it was not suitable for viral production. Despite this, Lulo can be considered a tool for understanding the mechanisms through which the cell can evade viral replication.

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# Erratum

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Felio Jesús Bello García

**Should read:**

Felio Jesús Bello