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MULTIPLICATION OF A TRANSMISSIBLE GASTROENTERITIS VIRUS CUBAN ISOLATE IN DIFFERENT CELL LINES

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ABSTRACT: **In this study we report a wide range of cells from different species, in which a Cuban TGE isolate multiplies. Both primary cultures and continuous cell lines from different species were used. We found out that 11 from the 13 kinds of cultures assessed were sensitive to the Cuban isolate, which not only showed an expected affinity to cells from porcine origin, but also it was able to replicate and produce a cytopathic effect in cells from bovine, hamster, monkey and quail origin.**

(Key words: transmissible gastroenteritis virus; porcine coronavirus; TGE; cellular multiplication; cytopathic effect).

MULTIPLICACIÓN DE UN AISLAMIENTO CUBANO DEL VIRUS DE LA GASTROENTERITIS TRANSMISIBLE EN DIFERENTES LÍNEAS CELULARES

RESUMEN: **En esta investigación reportamos un amplio rango de células de diferentes especies donde se multiplica un aislado cubano del virus de TGE, para lo cual se utilizaron tanto cultivos primarios como líneas celulares continuas, originadas de distintas especies animales. Se determinó que 11 de los 13 tipos de cultivos evaluados fueron susceptibles a la multiplicación del aislado cubano, el cual no mostró afinidad única por las células de origen porcino, ya que fue capaz de multiplicarse y producir efecto citopático en células de bovino, hámster, mono y codorniz.**

(Palabras clave: virus de la gastroenteritis transmisible del cerdo; coronavirus porcino; TGE; multiplicación celular; efecto citopático)

INTRODUCTION

Transmissible gastroenteritis of pigs (TGE) is a highly contagious disease, caused by a virus of the Coronaviridae family, Coronavirus genus (8). This disease is characterized by profuse diarrhoeas and vomiting (9), besides high mortality and lethality in suckling piglets (19, 13). Due to the serious losses it provokes in the pig industry, this disease is included in the list of disease of obligatory declaration of the World Organization for Animal Health (OIE) (11).

Coronavirus, as many animal viruses, is characterized by a restricted host range and tissue tropism (5, 6). Enjuanes and Cavanagh (7) report the cell lines: ST, PK 15 and LLC-PK1 (all from pig origin) to be permissible for TGE virus multiplication.

However, there are some reports of multiplication of this virus in canine and feline tissues (2).

Although some viruses are able to be multiplied in cells of a wide range of animal species, many of them are restricted to infect cells of their natural host species, since they require the presence of specific receptors for the virus attachments and penetration into the cells. TGEv entry into the host cells is mediated by the S glycoprotein through interactions with porcine aminopeptidase N (pAPN), which is the cellular receptor (15, 10).

APN, also called CD13, is a 150- to 160- Kda type II glycoprotein that is a membrane peptidase, which is expressed in cell surface of epithelial cells of kidney, intestine and respiratory tract; granulocytes,

monocytes, fibroblasts, endothelial cells, cerebral pericytes, at the blood-brain barrier and synaptic membranes in the Central Nervous System (CNS) (21).

In Cuba, TGE outbreaks with an epizootic presentation were reported (18), from which a virus isolate was obtained and cloned by plaque assay that is being currently characterized. Here we report the ability of this isolate to multiply in a wide range of cell lines of both mammal and bird origin.

MATERIALS AND METHODS

Virus: The 266c Cuban isolate of TGEv used throughout this study was obtained from piglets experimentally infected with homogenised intestinal tissue of sick animals (1) and cloned by plaque assay. Stock virus was passage 3 times in swine kidney cells (SK6) after cloning, with an infective titter of $10^{6.5}$ infective doses in tissue cultures ($IDTC_{50}$/ 0.1 ml).

Cells: We used both primary cultures and continuous cell lines from different species (Table 1).

Cell inoculation: 200 ìl of the viral stock were inoculated into each cell monolayer (previously washed with PBS) and 300 ìl of DMEM plus treated with 10 ìg/ ml trypsin and 20 mM Hepes (12) (DMEM HT). After incubation at 37ºC for 1 h, the cell sheets were overlaid with DMEM without trypsin.

Viral Neutralization: BHK-21 cell line was used for neutralizing the cytopathic effect with three monoclonal antibodies: 1DB12 (INGENASA, S.A, Spain), TGE25 c9.3c and TGE45 a8.f10.c6 (gently donated by the Dr. Osorio, University of Nebraska), all of them, against the S protein of the TGEv.

RESULTS AND DISCUSSION

With the exception of Fibroblast of Chicken Embryo (FCE) and MDCK cell line, the rest of the cells showed susceptibility to the multiplication of the virus evaluated (Table 2). The cytopathic effect found was characterized by rounded refringent cells, syncytia, detachment and lysis, plus final monolayer destruction in complete agreements with previous reports by Enjuanes and Cavanagh (7), who described the effect associated to this virus in swine cells. Uninfected cells were included which showed no effect.

Cowen and Braune (3) assessed the susceptibility of the QT-35 cell line regarding the multiplication of TGE virus. In this case no effect was found, thus showing that this cell line was not sensitive to the virus. However, our Cuban isolate did multiply in this cell line reaching the total destruction of the monolayer at 24 hours post-infection (pi) (Table 2).

Delmas *et al.* (4) accomplished the characterization of a surface molecule used by the virus to bind and enter the host cell. They report that aminopeptidase N acts as a membrane receptor suitable for viral attachment and penetration. These authors carried out recombinant aminopeptidase N expession in cells that under normal circumstances are not sensitive to TGEv multiplication. They used BHK-21 and MDBK cell lines, which have also been shown to be sensitive to the Cuban isolate multiplication, producing a complete cytophatic effect at 24 hours pi (Table 2, Figure 1).

TABLE 1. Types of cells assessed with the Cuban isolate of the TGE virus./ *Tipos de células evaluadas con el aislado cubano del virus de TGE.*

Cells	Type of Cultture	Organ	Species Origin	Source
RCe	Primary Culture	Kidney	Swine	CENSA
ST	Continuous Line	Testicle	Swine	University of Guelph, Canada.
SK6	Continuous Line	Kidney	Swine	University of Giessen, Germany.
PK-15	Continuous Line	Kidney	Swine	ATCC, CCL-33
RT	Primary Culture	Kidney	Bovine	CENSA
MDBK	Continuous Line	Kidney	Bovine	ATCC, CCL-12
BHK-21	Continuous Line	Kidney	Hamster	ATCC, CCL-10
BHH	Primary Culture	Heart	Hamster	CENSA
BHL	Primary Culture	Lung	Hamster	CENSA
VERO	Continuous Line	Kidney	Monkey	Institute of Animal Health, Pirbright.
QT-35	Continuous Line	Embryo	Quail	CIGB
FCE	Primary Culture	Embryo	Chicken	CENSA
MDCK	Continuous Line	Kidney	Canine	Biomedicum, Sweden, Uppsala.

TABLE 2. Cytopathic effect (CPE) ocurred in different cell lines evaluated./ *Efecto citopático (ECP) producido en las diferentes líneas celulares evaluadas*

Cells	Passages	Cytopathic effect (CPE)	CPE 100%
RCe	3 p	Yes	24 hours pi
ST	3 p	Yes	24 hours pi
SK6	3 p	Yes	24 hours pi
PK-15	3 p	Yes	24 hours pi
RT	1 p	Yes	72 hours pi
MDBK	2 p	Yes	24 hours pi
BHK-21	2 p	Yes	24 hours pi
BHH	1 p	Yes	96 hours pi
BHL	1 p	Yes	96 hours pi
VERO	2 p	Yes	24 hours pi
QT-35	1 p	Yes	24 hours pi
FEP	1 p	No	-
MDCK	1 p	No	-

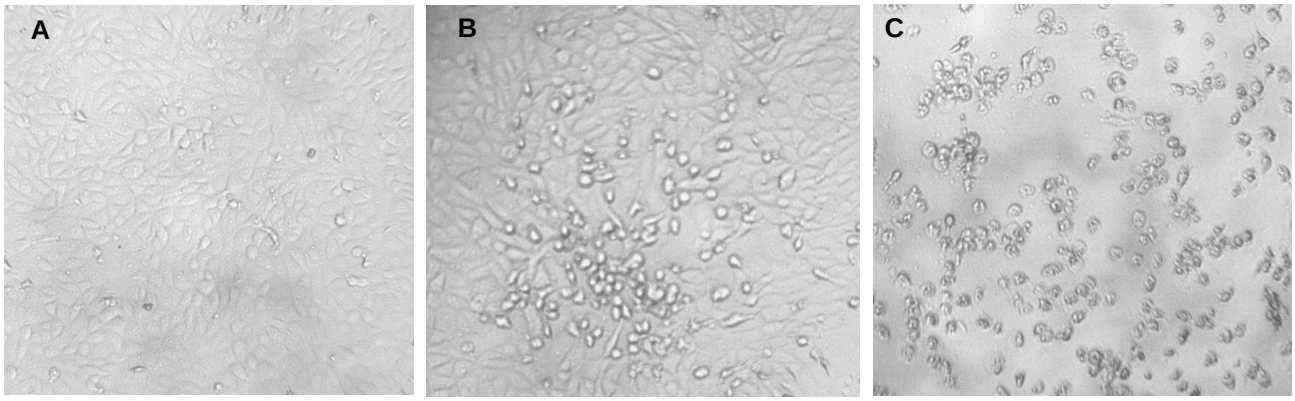

FIGURE 1. Cytopathic effect by the 266c Cuban isolate in the MDBK cell line.**A:** uninfected cells. **B:** infected cells at 12 hours PI. **C:** Infected cells at 24 hours pi./ *Efecto citopático provocado por el aislado 266c en la línea celular MDBK.* ***A:*** *Células sin infectar.* ***B:*** *Células Infectadas 12 horas PI.* ***C:*** *Células Infectadas 24 horas pi.*

Benbacer *et al.* (2) developed a similar study with BHK-21 and MDCK cell lines. Viral multiplication was attained once transfected cell expressed the specific receptor on their surface (aminopeptidase N). They report MDCK cell line as nonpermissive, in agreement with our results (Table 2). Nonetheless, even though we achieved viral identification before inoculating the different cell cultures, a viral neutralization assay was performed in BHK-21 cells with 3 monoclonal antibodies specifically reacting with viral protein S, wich showed a complete neutralization.

Coronaviruses attach to the host cells through the spike (S) glycoprotein. TGEv entry into the cells is also mediated by the S glycoprotein through interactions with porcine aminopeptidase N (pAPN), which is the cellular receptor. Aminopeptidase N also serves as the receptor for human, canine and feline coronaviruses (16, 17). These viruses have shown marked species specificity in receptor utilization, as Human Coronavirus (HCV-229E) can utilize human but not porcine APN, while TGEv can utilize porcine but not human APN. Thus, receptor specificity appears to be important determinant of the species specificity of HCV-229E and TGEv infection (20). Interestingly, while porcine and human aminopeptidases showed species specificity, the feline aminopeptidase (fAPN) seems to serve as a receptor for feline, canine, porcine and human coronaviruses (14).

The ability of nonfeline group coronaviruses to use fAPN as a receptor has important implications for the evolution of this group of viruses. These RNA viruses can undergo rapid evolution by recombiation. Possibly

recombination between different coronaviruses could ocurr in a cat that simultaneously infected with feline coronavirus (FeCV) or feline infectious peritonitis virus (FIPV) and another coronavirus in group I. Recombinants between different coronaviruses could have properties different from either parents and might show altered host range, tissue tropism, antigenicity, and/ or virulence, possibly resulting in emergence of a new disease (20).

Genetic alterations in either the virus or host cells can change the dynamics of virus-cell interaction. Retrospective studies showed that a domain of the spike protein encoded by S gene nucleotides (nt) 1518 to 2184 is efficiently recognized by pAPN (cell receptor), and transfection of pAPN to nonpermissive cells makes them susceptible to TGEv. In this study, they report that baculovirus-expressed polypeptides corresponding to amino-acids 522 to 744 of the spike protein were able to efficiently recognize pAPN (14).

According to the results obtained in this research, our isolate behaves in a different way as compared to previosly analyzed TGEv strains. This suggests possible changes at the genomic level, specifically in the region coding to the binding site virus-cell receptor. Therefore future studies will be aimed to accomplish a molecular study of the gene coding glycoprotein S.

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