# IN VITRO DIFFERENTIATION OF TRANSMISSIBLE GASTROENTERITIS VIRUS STRAINS BY PLAQUE SIZES IN SWINE TESTIS CELL CULTURE

P.P. Liou

**Characteristics of three transmissible gastroenteritis (TGE) virus field virulent strains (Gut origin: Ames #40, CN and Miller), three low cell culture-passaged strains (Ames #40-10, CN-10 and Miller-10), three high cell culture-passaged strains (SH-30, Neb-28 and Miller-60), and three vaccinal strains (SP, TO and Syntex) were studied to find the differentiation in plaque sizes in McClurkin swine testis cell culture among the four groups of virus strains. There were clear differences in plaque size when the strains were compared: The viruses of gut origin have plaque sizes ranging from 0.5 to 2.0 mm in diameters, low cell culture-passaged strains have plaque sizes ranging from 1.0 to 3.0 mm, high cell culture-passaged strains induced plaques larger than 3.0 mm, whereas vaccinal strains induced plaques that were smaller than 1.0 mm. The data suggest that the plaque method be useful as in vitro marker in the differentiation of TGE virus strains.**

Although it is known that in vivo biological differences between gut origin and cell culture-passaged transmissible gastroenteritis (TGE) virus strains are existence,[2,3,17] that is only little known about the properties of these strains in vitro.[14,15,16] In vitro there is lack of agreement about the discrepancies of TGE virus strains.[1,5,6,7,14,17] Hess and Bachmann[7] reported that there were clear differences in plaque size when the strains of TGE virus were compared. In contract to these results, some workers[1,5,18] could not find differences in plaque size between field isolates and cell culture-passaged strains.

The purpose of the present study was to compare the plaque pattern among the TGE strains. It is possible to evaluate the plaque patterns being one of the considerable value for the differentiation of TGE antigenical markers in vitro.

## MATERIALS AND METHODS

**Cell culture**: History and susceptibility of the McClurkin swine testis (ST)

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cell line to TGE virus have been reported [11] and this cell was used in this study. Cells were grown in a modified Earlés minimal essential medium (EMEM) supplemented with 10% fetal calf serum, 0.5% lactalbumin hydrolysate, 26.1 mM sodium bicarbonate and 50 μg/ml gentamicin sulfate, and they were cultured in 60 x 15 mm plastic petri plates. Confluent sheets of cells were obtained after 5 days of incubation at 37°C in a humidified atmosphere containing 5% $CO_2$.

**Viruses**: Three field virulent strains (gut origin), three vaccinal strains, three high cell culture-passaged strains were used. TGE field virus strains Were detected in ST cell cultures directly from intestinal suspensions of infected piglets. Field strains consisted of the Miller strain (piglet passage 3 times after original recovery, kindly supplied by L.J. Kemeny, Ames, Iowa); Chunan strain (CN, piglet passage 2 times after original recovery, isolated in Taiwan); and Ames #40 strain (piglet passage 1 time after original recovery, isolated in Iowa). In addition, the field strains of TGE virus serially passed 10 passed in ST cell culture were used and referred to as low cell culture-passaged strains (CN-10, Ames #40-10 and Miller-10). The three high cell culture-passaged strains consisted of the Nebraska strain (kindly supplied by R. D. Woods) after 28 passages in ST cell culture (Neb-28); SH strain (also supplied by R. D. Woods) after 30 passages in ST cells (SH-30), and a Miller strain which had been passaged 60 times in ST cells (Miller-60). The three vaccinal strains consisted of the TO strain[6] (kindly supplied by Y. P. Lin, Tansui, Taiwan), over 165 passages in primary swine kidney cell culture and 15 passages in ST cell culture, Syntex strain (cell culture vaccine, commercial product)**, and a small plaque strain[18] (Cultured continuously in ST cell cultures, kindly prepared the plaque plates by R. D. Woods, Ames, Iowa).

Cell culture infectivity titers of the viruses stated above were: Miller, $10^6$ plaque forming units (PFU) per ml; CN, $10^5$ PFU/ml; Ames #40, $10^7$ PFU/ml; Miller-10, $10^6$ PFU/ml; CN-10, $10^6$ PFU/ml; Ames #40-10, $10^6$ PFU/ml; Neb-28 $10^6$ PFU/ml; SH-30, $10^6$ PFU/ml; Miller-60, $10^7$PFU/ml:; TO, $10^{5.3}$ PFU/ml; Syntex, $10^{5.5}$ PFU/ml and SP, $10^7$ PFU/ml.

**Plaque formation**: Monolayer cultures were overlaid with 0.1 ml of virus suspension and kept in 37°C for 1 hour. The inoculum was removed after an adsorption time, and 7 ml of medium containing 1% agar was added. The petri dishes were incubated in a $CO_2$ atmosphere at 37°C for 48 hours, and then the cultures were fixed with absolute methanol and stained with 1% crystal violet in PBS. One hour later, the agar was poured away and plaques were detected. Each virus strain was inoculated onto different batches of ST cells to confirm the reproducibility of the plaque size.

## RESULTS

Field virus strains produced plaques with mean diameters varying from 0.5 to 2.0 mm (Fig. 1. B-1, B-2, B-3), vaccine strains induced plaques smaller than 1.0 mm (Fig. 1. A-1, A-2, A-3,), wh-

** TGE-Syntex, Lot No. 8045, Modified Live Virus Porcine Tissue Culture Origin, Des Moines, Iowa, USA.

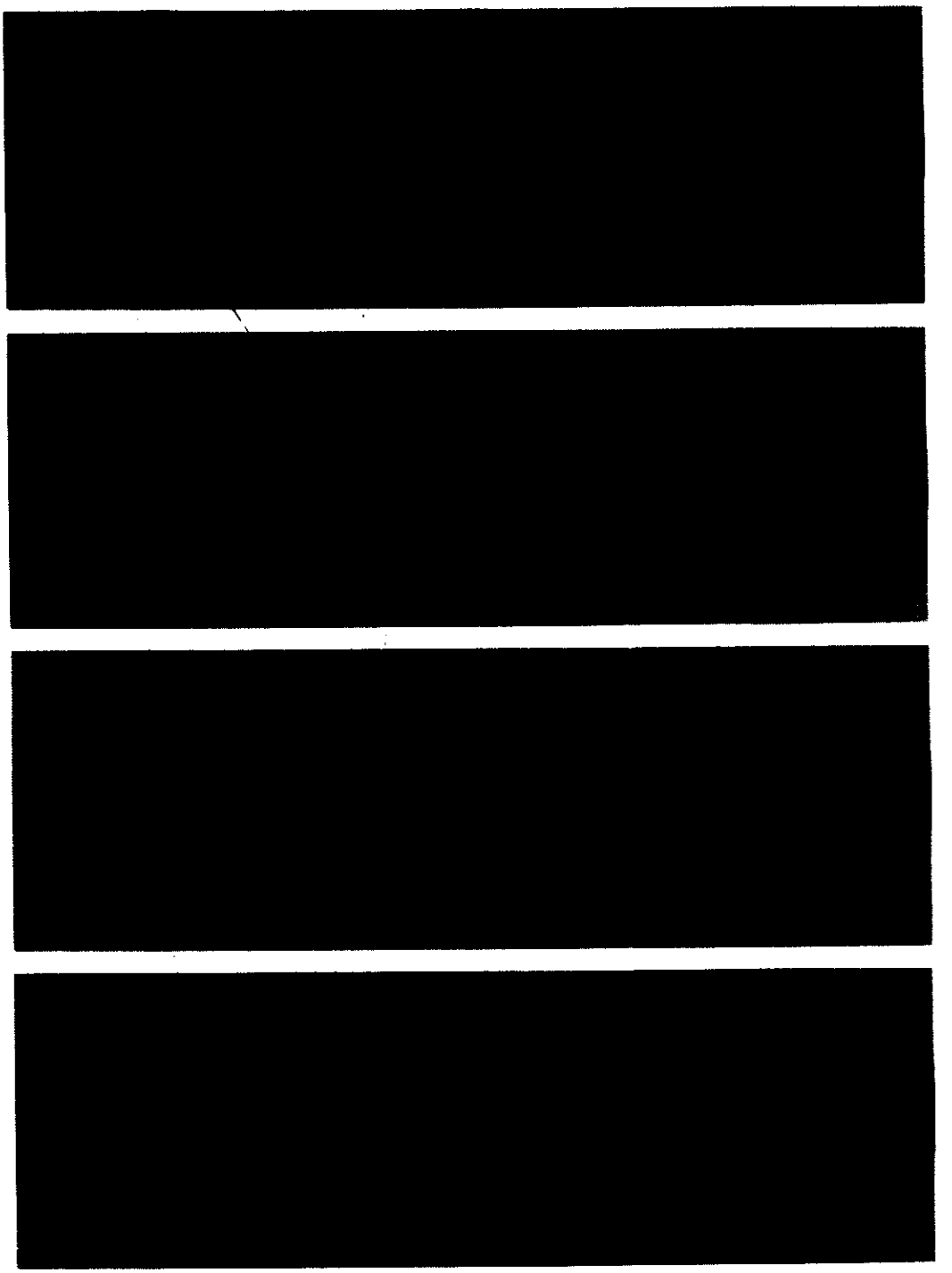

Fig. 1. Plaque morphologic characteristics of TGE viruses in McClurkin's swine testis cell monolayers. The plates were inoculated with viruses and incubated for 48 hours at 37°C. Plates were fixed with methanol and stained with 1% crystal violet.

Vaccinal Strains:
A-1 TO, A-2 Syntex, A-3 SP
Field virus strains:
B-1 CN, B-2 Ames #40, B-3 Miller
Low cell culture-passaged strains:
C-1 CN-10, C-2 Ames #40-10, C-3 Miller-10
High cell culture-passaged strains:
D-1 Neb-28, D-2 SH-30, D-3 Miller-60

ereas high cell culture passaged strains were larger than 3.0 mm (Fig. 1, D-1, D-2, D-3,) and varied only a little in diameters. Some variation in plaque size always could be observed in the low cell culture passaged TGE strains. These strains induced plaque sizes raning from 1.0-3.0 mm (Fig. 1. C-1, C-2, C-3).

## DISCUSSION

Although by in vitro cross-neutralization tests against different TGE viruses indicating that there was a close antigenic relationship among the viruses, [8] the viruses of gut origin had antigenic determinants not found in the cell culture propagated virus. [12,13] Apparently, cell culture derived virus has been modified in its antigenicity. [13] Antigenic change occur in TGE virus when the virus was passaged through cell culture. From these studies, it was demonstrated [4,13] that low cell culture passages of TGE virus had a minor antigenic change and high passages had a major antigenic change. The present study demonstrated that the TGE virus strains were different in plaque size (Fig. 1). It is suggested that the difference in plaque pattern among these strains be explained on the basis of population changes due to selective forces of cell culture passages. The discrepancies can also be observed by leukocyte-migration inhibition technique. [10]

Several references stated the discrepancies of TGE virus strains in plaque sizes without agreement, [1,5,7,18] this contradiction can perhaps be explained by the different susceptibility of cell cultures used for TGE viruses inoculation.

Plaque similarity between field virulent strains and vaccinal strains was shown (Fig. 1). The vaccinal strains used in present study elicited lactogenic immunity similar to that elicited by the virulent virus was studied by some researchers. [3,6,9,18] However, the limited observations suggest that there is a significant relationship between the antigenicity of TGE virus and plaque sizes induced in ST cell culture. The virus passaged in this cell culture and persistently induced the small plaque possibly be suitable for developing a vaccinal strain.

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# 於猪睪丸細胞形成斑灶作傳染性胃腸炎病毒株試管內鑑別

劉培柏

3株傳染性胃腸炎（TGE）病毒野外強毒（腸源：Ames #40，CN和Miller），3株細胞培養低繼代病毒（Ames #40-10，CN-10和Miller-10），3株細胞培養高繼代病毒（SH-30，Neb-28和Miller-60），以及3株疫苗病毒（SP，TO和Syntex），以此4組病毒於McClurkin猪睪丸細胞形成的斑灶特性作其鑑別試驗。由結果顯示各病毒株之斑灶大小有顯著不同：腸源病毒之斑灶大小爲直徑0.5至2.0 mm之間，細胞培養低繼代病毒之斑灶大小爲1.0至3.0 mm之間，細胞培養高繼代病毒之斑灶大於3.0 mm，而疫苗病毒所形成之斑灶則小於1.0 mm。猜測斑灶法可作爲TGE病毒株於試管內之鑑別指標。

台灣省家畜衞生試驗所