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Comparative infection efficiency of PRRSV field isolates on MA-104 cells or porcine alveolar macrophages

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Introduction

Since the recognition of Porcine Respiratory and Reproductive Virus (PRRSV) most viral isolations in North America have been carried in continuous cell lines derived from green monkey kidney epithelial cells (MARC-145, MA-104 or Cl-2621 cells). Porcine pulmonary alveolar macrophages (PPAM) are used less often even though they are a naturally permissive host cell and wild-type PRRSV field isolates were described that did not grow on MA-104 cells[1]. It is though that for routinely field isolation it is difficult to obtain sufficient number of PPAM cells. In the course of a study of genetic variation in field viruses we observed that some isolates grew better on PPAM than on MA-104 cells. Therefore, we conducted a more thorough study to characterize the growth of field isolates on primary PPAM and MA-104 cells.

Objectives

1. To isolate and store a large population of PPAM from 5 to 6 week-old piglets for future use.
2. To determine if porcine pulmonary alveolar macrophages were suitable for routine isolation of PRRSV field strains compared to MA-104 cells.

Material and Methods

Cells: PPAM were collected from 7 healthy 5-to-6 week old piglets. The MA-104 cell line was obtained from the American Type Culture Collection (Bethesda MD). Fifty serum samples were collected from farms where a typical outbreak of PRRS had occurred. Cells in a 24 well plate were inoculated with 300 µl of serum, left at room temperature for 1h, then incubated at 37°C in a 5 % humidity CO_2 incubator for 5 days. A VR-2332 PRRSV laboratory adapted strain was used as positive control. Immunofluorescence with SDOW17 monoclonal antibody to nucleocapsid and quantitative reverse transcriptase-PCR were performed to assess viral infection status.

Results

Lung lavage from 7 pigs generated an average of $2.3x10^9$ PPAM in 100 ml, with 99% viability (Table 1). After freezing in liquid nitrogen, plated cells were more than 90% macrophages.

Table 1. Pulmonary lavage from 7 piglets.

No. of Pig	PBS (ml)	Recovered PBS (ml)	PPAM/ ml
1	1000	600	$2x\ 10^7$
2	1000	750	$8x\ 10^7$
3	1000	700	$2X\ 10^7$
4	1000	500	$1\ X\ 10^7$
5	1000	650	$2X10^6$
6	1000	750	$3\ x\ 10^7$
7	1000	700	$5\ x\ 10^6$
Average		665	$2.3\ x\ 10^7$

All lots of PPAM collected support PRRSV growth

Field PRRSV was isolated as early as 72 h post-inoculation in PPAM, when virus aggregates were observed in cytoplasm with SDOW-17 antibody. Twenty (40%) sera showed CPE. But only eighteen samples (38%) were SDOW-17 and RT PCR (Ct < 38) PRRSV positive on PPAM. One field strain grew also in MA104 cells (Table 2).

Table 2. Field PRRSV growth results.

	MA 104		PPAM		SDOW17		RT-PCR	
	CPE	No CPE	CPE	No CPE	(+)	(-)	Ct<38 (+)	Ct>38 (-)
50	1	49	20	30	18	30	33	15
%	2	98	40	60	38	63	69	31

CPE= cytopathic effect

Discussion and Conclusions

In the beginning the isolation of PRRSV field strain from pig serum samples was performed in MA-104 cells, but we learned that the majority of the samples were negative for PRRSV even though the samples came from endemically infected sow herds and some were PCR positive. Similar data were obtained by Bautista et al[1], who concluded that although PAM were relatively more sensitive for PRRSV both PPAM and CL-2621 should be used for a high rate of PRRSV isolation. We recovered 20 field isolates in PPAM versus one isolate in MA-104. The VR2332 strain, widely used as a reference strain, grew efficiently in MA-104 but poorly in alveolar macrophages. We conclude that for PRRSV field virus isolation PPAM is superior.

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