

Is the PRRSV isolate obtained in cell culture the same as the virus present in the original clinical sample?

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Introduction

Isolation of porcine reproductive and respiratory syndrome virus (PRRSV) in cell culture is a primary means of obtaining virus isolates for autogenous vaccine production and other applications. The objective of this study was to determine whether cell culture isolate and the virus in the clinical sample are sequence equivalent or not.

Materials and Methods

In total, 1024 PRRSV PCR-positive (995 PRRSV-2, 26 PRRSV-1, and 3 co-infected PRRSV-1 and PRRSV-2 PCR-positive) clinical samples and their isolates obtained in MARC-145 and/or ZMAC cells during 2010-2020 were included in this study. ORF5 sequences of 1024 clinical samples, 837 MARC-145 isolates (passage 0 or P1), and 270 ZMAC isolates (P0 or P1) were determined and compared for RFLP patterns, genetic lineages, and nucleotide identities. For those cases with non-matching PRRSV between clinical sample and cell culture isolate, next-generation sequencing (NGS) and vaccine-specific PCR were conducted to elucidate the differences.

Major findings and implications

- PRRSV virus isolation (VI) success rate was significantly higher in ZMAC than in MARC-145 cells for serum and lung samples containing PRRSV-1, PRRSV-2, or PRRSV-1 & PRRSV-2 co-infection.
- For clinical samples evaluated in this study (3 positive for both PRRSV-1 and PRRSV-2, 26 PRRSV-1, and 96.2% [957/995] of PRRSV-2), the predominant ORF5 sequences of PRRSV in the clinical samples and the respective cell culture isolates were matching in regards to RFLP patterns, genetic lineages, and nucleotide identities (Table 1).
- Small percentage of PRRSV-2 PCR-positive clinical samples (2.4%, Category 2) and their MARC-145 and/or ZMAC isolates had 98.6-99.8% ORF5 nucleotide identity and the same genetic lineages but different RFLP patterns due to point mutation(s) located at the *HincII* or *SacII* restriction site, but were considered as the same virus strains (Table 1).
- In the PRRSV-2 Category 3 (14/995; 1.4%), the predominant PRRSV-2 ORF5 sequences derived directly from clinical samples were different from those from their corresponding MARC-145 isolates but were similar to their corresponding ZMAC isolates (Table 1). In those cases, most isolates obtained in MARC-145 cells contained Ingelvac PRRS MLV vaccine-like virus while the predominant viral sequences detected in clinical samples and ZMAC isolates were wild-type strains. This is concerning because autogenous vaccines produced from MARC-145 isolates may not contain the desired wild-type virus strain found on the farm.
- Vaccine-specific PCR and NGS performed on selected cases in PRRSV-2 Category 3 confirmed presence of ≥ 2 PRRSV-2 strains (mixed infection) in such clinical samples. In co-infected samples, while Sanger sequencing determines the predominant strain ORF5 sequence from the clinical sample (i.e. wild-type A), if wild-type strain A has lower growth adaptability or kinetics compared to the other strain (i.e. vaccine-like strain B) in MARC-145 cells, vaccine-like strain B could be isolated in MARC-145 cells although wild-type strain A is isolated in ZMAC cells.
- Characterizing PRRSV sequences from clinical samples and cell culture isolates should be conducted before using isolates for producing autogenous vaccines or other applications.

Table 1. Summary of virus isolation results and comparison between virus isolates and clinical samples for PRRSV-1 and PRRSV-2

Type	MARC-145 VI+	MARC-145 VI-	MARC-145 ND ¹	Total (%)	RFLP	Lineage	ORF5 nt identity ²
PRRSV-1	ZMAC VI+	5	14	1	20	N/A ³	N/A
	ZMAC VI-	0	0	0	0		
	ZMAC ND	6	0	0	6		
	Total	11	14	1	26 (100%)		
PRRSV-2 (Category 1)	ZMAC VI+	61	134	34	229	Consistent ⁵	Consistent ⁵
	ZMAC VI-	7	0	0	7		
	ZMAC ND	721	0	0	721		
	Total	789	134	34	957 (96.2%) ⁴		
PRRSV-2 (Category 2)	ZMAC VI+	2	4	1	7	Inconsistent ⁶	Consistent ⁵
	ZMAC VI-	0	0	0	0		
	ZMAC ND	17	0	0	17		
	Total	19	4	1	24 (2.4%) ⁴		
PRRSV-2 (Category 3) ⁷	ZMAC VI+	6	0	0	6	Consistent (ZMAC);	Consistent (ZMAC);
	ZMAC VI-	0	0	0	0	Inconsistent (MARC-145)	Inconsistent (MARC-145)
	ZMAC ND	8	0	0	8		
	Total	14	0	0	14 (1.4%) ⁴		

1. ND: Virus isolation was not done. 2. ORF5 nucleotide identities between clinical samples and their isolates.

3. N/A: Not applicable. 4. (%) was calculated based on the number of PRRSV-2 samples in each category and the total sample number of 995.

5. Consistent: Results were similar between PRRSV-2 PCR-positive clinical samples and isolates in MARC-145 and/or ZMAC.

6. Inconsistent: Results were different between PRRSV-2 PCR-positive clinical samples and isolates in MARC-145 and/or ZMAC.

7. For PRRSV-2 Category 3, the PRRSV isolate in ZMAC cells was matching with the predominant virus strain in the clinical sample whereas the PRRSV isolate in MARC-145 cells was not matching with the predominant virus strain in the clinical sample.