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Field Application of a Monoclonal Antibody Panel to Differentiate Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Isolates

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Porcine reproductive and respiratory syndrome (PRRS) is recognized as the most economically devastating disease of swine throughout the world. Nucleotide sequencing and serological studies have demonstrated substantial antigenic variation among PRRS virus (PRRSV) isolates (Murtaugh, et al., 1995; Nelson, et al., 1993, 1996). Recently, European-like PRRSV isolates have been identified in the US (Rossow, et al. 2000) and other new, highly virulent PRRSV isolates have appeared and affect sows at all stages of gestation causing mortality of adult pigs as well as young pigs (Epperson et al., 1997). Therefore, it is even more important to control the spread of these new strains. Clinical signs vary between herds, indicating that viruses may differ in pathogenicity. Furthermore, effective use of vaccines and management approaches to control PRRS has not always been successful.

Pigs previously infected with one strain of PRRSV or vaccinated with available modified-live vaccines may not necessarily be completely protected against challenge by all other isolates or strains of PRRSV. Therefore, producers are concerned about introducing new, possibly more virulent forms of the virus into already infected herds. Producers are also using various acclimation protocols to introduce new replacement animals to PRRSV strains present in a herd to avoid major outbreaks during gestation and farrowing. Pork producers have an expressed interest in knowing which strains of PRRSV are circulating in their herds. This information is then used to assist in decisions regarding acclimation and production and use of autogenous vaccines. Currently, the primary means of strain differentiation involves nucleotide sequencing of selected regions of the PRRSV genome following isolation of the virus.

There is little understanding of how observed sequence differences between isolates actually translate to significant antigenic differences that influence how the virus affects an animal. Antigenic epitopes recognized by monoclonal antibodies (MAbs) represent structural differences in the viruses rather than just nucleotide substitutions that may have no effect on virus structure or recognition by the host immune system.

The objectives of this study were 1) To develop a marker system based on epitopic differences among PRRSV isolates that will allow for the rapid differentiation of PRRSV isolates, and 2) To evaluate the application of this marker system in large swine production systems.

(Key Words: PRRSV, antigenic variation, monoclonal antibodies)

Experimental Procedures

Collaborative studies at Iowa State University and South Dakota State University have resulted in a large panel of 60 MAbs representing the products of PRRSV open reading frames (ORFs) 2 through 7 (Nelson, et al., 1996, Yang, et al., 1999). This panel of MAbs was used in an indirect fluorescent antibody (IFA) assay to categorize PRRSV isolates into distinct groups (Yang, et al. 1999). To date, over 400 American field isolates, the European Lelystad virus and four modified-live vaccine strains have been evaluated. Seventy distinct reactivity patterns were identified among these virus isolates (Jones, et al. 1999). This assay can clearly differentiate European-like isolates from North American isolates and can differentiate two of the previously available

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modified-live vaccine strains from field isolates, as well as identify many antigenic differences among other field isolates (Table 1). We then evaluated the field application of this MAb panel in an effort to categorize PRRSV isolates by antigenic variability. To do this, we conducted a field survey of 259 PRRSV isolates, followed by a survey of additional 136 isolates from two large swine production systems.

Results

The 259 virus isolates in the initial field survey were categorized into 65 distinct groups based on differences in MAb reactivity patterns. Five additional groups, not previously detected, were identified in one of the production systems (Table 2). A total of 18 different MAb reactivity patterns were identified among the 93 isolates from Production System #1. Six different patterns were identified among the 42 isolates from System #2. The distribution of MAb reactivity patterns among isolates from System #1 was similar to that of the initial field survey. However, the distribution of patterns among isolates from System #2 was very different. Approximately 79% of the isolates from System #2 were unique to that production system and were not identified in the field survey or System #1 (Table 2).

PRRSV isolates were obtained from 31 different sites within Production System #1. Isolates with three different MAb reactivity patterns were identified from two of these sites, suggesting circulation of multiple strains or forms of the virus at a single site. Two distinct MAb reactivity patterns were apparent in each of 10 sites and only one pattern was identified in each of the remaining 19 sites.

Summary

This work demonstrates that MAbs can be used to categorize PRRSV isolates based on antigenic variability. This MAb panel can provide another tool, beyond sequence analysis, by detecting structural differences in the PRRSV proteins for the differentiation of isolates. Substantial antigenic variation exists among North American PRRSV isolates, as demonstrated by the identification of 70 distinct MAb reactivity patterns and multiple PRRSV isolates showing antigenic variation were identified from single production sites. This MAb panel may provide a valuable marker system for epidemiological studies, as well as providing a tool to assist producers with decisions regarding animal movement, acclimation protocols and the selection of isolates for autogenous vaccine production. In the future, we may be able to establish correlations between serological groups with respect to factors such as virulence, cross-protection and other biological functions.

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TABLE 1. DIFFERENTIATION OF SELECTED MODIFIED-LIVE VACCINES AND EUROPEAN PRRSV ISOLATES FROM AMERICAN FIELD ISOLATES USING FOUR MAbs TO THE NUCLEOCAPSID PROTEIN.

	Monoclonal Antibody			
Isolate/Vaccine	SR30	SDOW17	MR40	JP24
US Field Isolates	+	+	+	+
Ingelvac PRRS® MLV	+	+	+	+
Prime Pac® PRRS	+	-	+	+
Suvaxyn® PRRS	+	+	-	+
European isolates	+	+	-	-

TABLE 2. DISTINCT MAb REACTIVITY PATTERNS OBSERVED IN A FIELD SURVEY AND IN TWO SEPARATE U.S. SWINE PRODUCTION SYSTEMS.

	Number of Isolates		
*Group	Field Survey	System #1	System #2
1	66 (25.6%)	30 (32.3%)	9 (20.9%)
2	60 (23.3%)	19 (20.4%)	0 (0.0%)
3	22 (8.5%)	6 (6.5%)	0 (0.0%)
4	12 (4.6%)	6 (6.5%)	0 (0.0%)
5	7 (2.7%)	3 (3.2%)	0 (0.0%)
6	6 (2.3%)	0 (0.0%)	0 (0.0%)
7	5 (1.9%)	0 (0.0%)	0 (0.0%)
8	5 (1.9%)	0 (0.0%)	0 (0.0%)
9	5 (1.9%)	2 (2.2%)	0 (0.0%)
10	3 (1.2%)	1 (1.1%)	0 (0.0%)
11	3 (1.2%)	0 (0.0%)	0 (0.0%)
12	3 (1.2%)	0 (0.0%)	0 (0.0%)
13	2 (0.8%)	1 (1.1%)	0 (0.0%)
14	2 (0.8%)	0 (0.0%)	0 (0.0%)
15	2 (0.8%)	0 (0.0%)	0 (0.0%)
16	2 (0.8%)	11 (11.8%)	0 (0.0%)
17	2 (0.8%)	0 (0.0%)	0 (0.0%)
18	2 (0.8%)	0 (0.0%)	0 (0.0%)
19	2 (0.8%)	1 (1.1%)	0 (0.0%)
20	2 (0.8%)	1 (1.1%)	0 (0.0%)
21	2 (0.8%)	0 (0.0%)	0 (0.0%)
22-64	42 (16.2%)	12 (12.9%)	0 (0.0%)
65-69	0 (0.0%)	0 (0.0%)	34 (79.1%)
70 (Lelystad)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Total	259	93	43

*MAb reactivity groups 1-64 were originally defined by Yang, et al., 3rd International Symposium on PRRSV, Ploufragan, France. Groups are numbered in descending order based on the number of isolates showing a specific reactivity pattern in the initial field survey. Groups 65-69 were only identified in Production System #2. Group 70 represents the European Lelystad isolate.