Antigenic Comparison of Canadian and US Isolates of Porcine Reproductive and Respiratory Syndrome Virus Using Monoclonal Antibodies to the Nucleocapsid Protein

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ABSTRACT

Fifteen Canadian field isolates of porcine reproductive and respiratory syndrome (PRRS) virus from Quebec and Ontario were compared with 5 US PRRS virus (PRRSV) isolates and with the European Lelystad isolate using monoclonal antibodies (MAbs) SDOW17, EP147, and VO17 directed to the 15-kDa nucleocapsid protein of PRRSV. All Canadian and US isolates tested by indirect immunofluorescence were recognized by the 3 MAbs, and individual titers of MAbs towards Canadian and US PRRSV isolates were similar as well. In contrast, the Lelystad virus isolate reacted only with the SDOW17 MAb and showed no reactivity with either EP147 or VO17. The reactivity pattern with these MAbs suggests that the Canadian isolates of PRRSV tested are antigenically similar to US isolates of PRRSV, and that these North American isolates share highly conserved epitopes on the 15-kDa nucleocapsid protein that clearly differentiate them from the European Lelystad virus isolate.

RÉSUMÉ

Quinze isolats canadiens du virus du syndrome reproducteur et respiratoire du porc (SRRP) provenant du Québec et de l'Ontario ont été comparés à 5 isolats des États-Unis et à l'isolat européen de Lelystad à l'aide des anticorps monoclonaux (AcMo) SDOW17, EP147 et VO17, dirigés contre la protéine de la nucléocapside ayant un poids moléculaire de 15-kDa. Tous les isolats du Canada et des États-Unis testés par immunofluorescence indirecte ont été reconnus par les 3 AcMo. Les titres individuels des AcMo envers les isolats éprouvés étaient également similaires. Par contre, l'isolat Lelystad du virus SRRP démontra une réactivité seulement avec l'AcMo SDOW17, mais aucune avec les AcMo EP147 et VO17. Le type de réactivité obtenu avec ces AcMo suggère que les isolats du virus SRRP du Canada testés sont antigéniquement similaires à ceux des États-Unis et que ces isolats nord-américains partagent des épitopes hautement conservés sur la protéine de la nucléocapside de 15-kDa qui les différencient nettement de l'isolat européen de Lelystad.

Porcine reproductive and respiratory syndrome (PRRS) is a new viral disease of swine caused by a small, nonhemagglutinating, RNA virus (1,2) having properties of the *Arterivirus* group (3,4). Antigenic variations have been reported between European and US isolates of PRRS virus (PRRSV), and antigenic differences have also been observed among US isolates (5). In a serologic survey for European Lelystad and US VR-2332 strains of PRRSV, evidence for the US swine herds being affected with a Lelystad-like strain was found (6). More recently PRRSV isolates of the US and Europe have been shown, using monoclonal antibodies (MAbs) to the 15-kDa nucleocapsid protein, to contain both conserved and divergent epitopes on this protein (7). In that study, 4 of the MAbs evaluated reacted with all US isolates tested, but failed to react with PRRSV isolates of several European countries, including the Lelystad virus isolate, thus establishing the distinctiveness between US and European isolates of PRRSV. Although some of these MAbs have been used in the identification of Canadian isolates of PRRSV (8–14), little information exists on the degree of antigenic relatedness between Canadian and US isolates of this virus. The objective of the present study was to compare Canadian and US isolates of PRRSV in order to appreciate this antigenic relatedness.

Twelve isolates of PRRSV from Quebec and 3 from Ontario were compared with 5 US isolates of PRRSV and with the Lelystad virus. Quebec isolates LHVA-92-2 (9), LHVA-93-3 (13), LHVA-94-7, KLOP, BAJ, DESR, SBC (12), 93-085, 93-653, 93-2616-13, 94-3282-16, and 94-2072, and Ontario isolates LHVA-93-4, LHVA-93-5, and LHVA-93-6 (11) were originally isolated on porcine alveolar macrophages and further propagated on MARC-145 cells (15). The reference US isolate ATCC-VR-2332 and SD1 were isolated on CL2621 and ST cells, respectively (2,7), and US isolates 91-23983, 91-22778, and 91-1453B were isolated on porcine alveolar macrophages. The US isolates and the Lelystad isolate (1) were also further propagated on MARC-145 cells. Three PRRSV MAbs to the 15-kDa protein (7) were used in the present study: SDOW17, EP147, and VO17. Indirect immunofluorescence was used to titrate each of the 3 MAbs against all isolates. Briefly, MARC-145 cells infected with the different isolates were prepared in 96-well microtiter plates and fixed in cold acetone. Two-fold dilutions in phosphate buffered saline

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Table I. Indirect immunofluorescence titers of MAbs SDOW17, EP147, and V017 to Canadian, US, and the Lelystad isolates of PRRSV

PRRSV isolate	Titers[a]		
	SDOW17	EP147	VO17
LHVA-92-2[b]	51 200	1600	102 400
LHVA-93-3[b]	51 200	1600	102 400
LHVA-93-4[b]	51 200	1600	102 400
LHVA-93-5[b]	51 200	1600	102 400
LHVA-93-6[b]	51 200	1600	102 400
LHVA-94-7[b]	51 200	800	102 400
KLOP[c]	51 200	1600	102 400
DESR[c]	25 600	1600	51 200
SBC[c]	51 200	800	51 200
93-085[c]	51 200	1600	102 400
93-653[c]	102 400	1600	51 200
93-2616-13[c]	51 200	1600	102 400
94-3182-16[c]	102 400	1600	102 400
94-2072[c]	102 400	1600	102 400
94-287[c]	102 400	1600	51 200
VR-2332[d]	102 400	1600	102 400
SD-1[d]	25 600	3200	51 200
91-23983[d]	25 600	1600	51 200
91-22778[d]	25 600	1600	51 200
91-1453B[d]	51 200	3200	102 400
Lelystad[b,c,d]	51 200–102 400	<100	<100

[a] Titers of PRRSV MAbs by indirect immunofluorescence expressed as the highest dilution at which specific cytoplasmic fluorescence was observed

[b] Isolate tested at Laboratoire d'hygiène vétérinaire et alimentaire, Agriculture Canada, Saint-Hyacinthe (Québec), Canada

[c] Isolate tested at Centre de virologie, Institut Armand-Frappier, Laval (Québec), Canada

[d] Isolate tested at Department of Veterinary Science, South Dakota State University, Brookings, South Dakota, USA

(PBS) were prepared for each MAb and added to duplicate wells of infected and noninfected cells. Following an incubation of 30 min at 37°C and washings in PBS, fluorescein-conjugated antimouse IgG was added to the wells and incubated for 30 min at 37°C. After final washings, the plates were read using a fluorescence microscope and titers determined as the reciprocal of the highest dilution of the MAb giving specific cytoplasmic fluorescence.

The results obtained from the 3 different laboratories and compiled in Table I show that comparable titers were obtained for the SDOW17 MAb with all Canadian and US isolates of PRRSV tested, as well as with the Lelystad isolate of PRRSV. Similar results towards Canadian and US isolates were also obtained with the VO17 MAb but, as previously reported (7), this MAb did not react with the Lelystad isolate. The EP147 MAb also did not react with the Lelystad isolate but reacted with all Canadian and US isolates, albeit at lower titers than did the VO17 MAb.

Based on the reactivity of these 3 MAbs directed at epitopes on the 15-kDa nucleocapsid protein of PRRSV, the Quebec and Ontario PRRSV isolates were antigenically quite similar. The reactivity patterns were also very similar to the US isolates tested, but different than that of the Lelystad isolate of PRRSV. Although only 1 European isolate was tested in the present study, it has been previously reported that PRRSV from various countries in Europe reacted with these MAbs similarly to the Lelystad virus (7). The results of the present work further indicate that these MAbs recognize epitopes on the nucleocapsid protein that are highly conserved amongst North American isolates. Although serological data suggest the existence of Lelystad-like isolates in the US (6), the present study of several Canadian isolates of PRRSV, collected from different geographical locations and following different clinical outbreaks of disease, could not identify any isolate as a Lelystad-like PRRSV. Studies are in progress to identify and characterize any antigenic variations, among Canadian isolates, on other structural proteins having determinants involved in biological functions such as virus neutralization.

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