

Viral infection of the monocyte-macrophage system of this pigeon demonstrated by both light and transmission electron microscopy suggests that the virus may interfere with antigen processing in pigeons, as is speculated in psittacine birds with PBF. Multiple infections with bacteria (*Chlamydia psittaci*, *Pasteurella* sp., and an enteropathogenic coccobacillus), parasites (*Tetrameres* sp., and *Trichomonas* sp.), and viruses (presumptive paramyxovirus or herpesvirus) suggest that acquired immunodeficiency was associated with the circovirus-like agent.

Immunohistochemistry, DNA *in situ* hybridization, and polymerase chain reaction with DNA dot-blot hybridization indicate that this pigeon circo-like virus is distinct from PBF virus with respect to antigenicity and nucleic acid sequence. However, both viruses are similar with respect to suggestion of acquired immunodeficiency and association with a plethora of secondary infections.

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Serologic survey for Lelystad and VR-2332 strains of porcine respiratory and reproductive syndrome (PRRS) virus in US swine herds

Elida M. Bautista, Sagar M. Goyal, James E. Collins

The porcine reproductive and respiratory syndrome (PRRS), a disease that has been affecting swine herds in North America since 1987⁵ and in Europe since 1990,^{3,10,11} has gained worldwide attention because of its devastating economic impact on the pig industry.⁷ The disease is caused by a virus referred to as the Lelystad virus in Europe¹⁰ and as the ATCC VR-2332 strain of PRRS virus in the USA.²

The antigenic comparison of PRRS virus isolates has indicated the existence of strain differences among PRRS viruses, especially among the American isolates.⁹ Also, some PRRS virus isolates grow exclusively in 1 or the other of the 2 available cell systems (porcine alveolar macrophages or a stable cell line CL2621), whereas some other isolates grow equally well in both cell systems? Additional evidence of strain variation can be found in a recent report in which

animals seropositive to Lelystad virus were found to be negative when tested for VR-2332 antibody (J. E. Pearson, personal communication).

The important implications of the above findings prompted a study to determine the prevalence of antibodies to both Lelystad and VR-2332 strains of PRRS virus in the US pig population. For this purpose, a total of 837 porcine serum samples obtained from 18 different states were tested. Of these 837 samples, 456 were from 48 different farms and were submitted to the Minnesota Veterinary Diagnostic Laboratories from April 1992 to October 1992 for the detection of PRRS virus antibodies. The remaining 381 serum samples were from 39 farms and were collected as a part of the National Animal Health Monitoring System (NAHMS) survey of breeding animals located in 18 different states.⁶

In addition, 4 experimental sera were used: 1) serum from 2 conventional pigs experimentally infected with VR-2332, collected before infection and after 3, 7, 14, 21, 28, 35, and 42 days postinfection (dpi); 2) serum from a gnotobiotic piglet inoculated with the Lelystad virus, collected at 56 dpi;^a 3) serum from caesarian-derived, colostrum-deprived pigs in-

From the Department of Veterinary Diagnostic Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108. Address reprint requests to Dr. Goyal.

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Table 1. Occurrence of anti-Lelystad (LV) and anti-VR-2332 (VR) antibodies in 837 pig sera from 87 farms.

Antibodies*	Pigs		Farms	
	No.	%	No.	%
VR-/LV-	358	42.8	15	17.2
VR-/LV+	96	11.5	7†	8.1
VR+/LV-	210	25.1	14	16.1
VR+/LV+	173‡	20.6	51	58.6
Kappa value	0.246		0.422	

* - = negative at 1:16; + = positive at \geq 1:16.

† Of these 7 farms, 3 were in Minnesota, 2 in Alabama, and 1 each in Kansas and Colorado.

‡ Of these 173 samples, 41 had the same titers, 10 had lower titers to VR-2332, and 122 had higher titers to VR-2332.

oculated with VR-2332, collected at 63 dpi;^a and 4) serum from conventional pigs inoculated with the Lelystad virus, collected at 39 dpi.^b

All serum samples were tested for antibody to both the Lelystad and the VR-2332 strains of PRRS virus by an indirect fluorescent antibody (IFA) test.¹ Three-day-old CL2621^c cell monolayers prepared in 96-well microtiter plates were infected with 1,000 TCID₅₀ per well of VR-2332 (passage 7 in CL2621 cells) or Lelystad virus (passage 10 in porcine alveolar macrophages followed by 5 passages in CL2621 cells). After 48-72 hours in incubation, the plates were fixed with cold absolute ethanol and stored at -20 C until used (usually 2-4 weeks). Four-fold dilutions (1: 16 to 1: 1,024) of sera were prepared in microtiter plates, and 0.1 ml of each dilution was transferred to virus-infected cells. After incubation at 37 C for 45 minutes, unbound antibodies were removed by washing 3 times with phosphate-buffered saline (pH 7.2) followed by the addition of 50 μ l per well of a fluorescein-conjugated anti-porcine IgG.^d After another incubation for 45 minutes, the plates were washed as above, dried, and observed with a fluorescent microscope for specific cytoplasmic and perinuclear fluorescence.^{4,12} A positive^a and a negative control were included in each plate. A titer of L 1: 16 was considered positive.^{1,12}

All 4 experimental sera reacted with their respective homologous strains at titers of \geq 1:2,048. Antisera against VR-2332 and Lelystad viruses, obtained at 39-42 dpi, reacted only with their homologous strains and not with heterologous strains. However, anti-Lelystad serum, obtained at 56 dpi, reacted with VR-2332 at a titer of 1: 1,024. Similarly, anti-VR-2332 serum, obtained at 63 dpi, had a titer of 1:256 against the Lelystad virus. In another study, using the immunoperoxidase monolayer assay, anti-Lelystad antibodies were not detectable in serum prepared against VR-2332 but anti-VR-2332 antibodies were present in anti-Lelystad serum.⁹ These results suggest that although there are differences between the VR-2332 and the Lelystad strains of PRRS, they do share some common antigenic determinants that induce antibody responses to heterologous strains later in infection. However, further studies are needed to confirm these observations.

The results in Tables 1 and 2 show that 358 (42.8%) of the 837 samples were negative for antibodies to both strains

Table 2. Indirect fluorescent antibody titers to Lelystad (LV) and VR-2332 strains of PRRS* virus (kappa value = 0.129).

LV†	VR-2332†					Total
	0	16	64	256	1,024	
0	358	18	47	56	89	568
16	26	1	9	11	20	67
64	47	2	5	15	27	96
256	12	0	3	13	40	68
1,024	11	0	1	4	22	38
Total	454	21	65	99	198	837

* PRRS = Porcine reproductive and respiratory syndrome.

† Reciprocal of antibody titers.

of PRRS virus, whereas 479 (57.2%) were positive for antibodies to 1 or both strains. Of the 479 antibody-positive samples, 96 (20.1%) were positive for the Lelystad strain only, 210 (43.8%) were positive for the VR-2332 strain only, and 173 (36.1%) were positive for both strains (Table 1). Of the 173 samples that had antibody to both strains, 41 (23.7%) had the same titers for both viruses, 122 (70.5%) had higher titers with the VR-2332 strain, and 10 (5.8%) had higher titers with the Lelystad strain (Table 2). The statistical analysis of data obtained with these 837 samples indicated poor correlation between the VR-2332 and the Lelystad strains of PRRS virus ($\kappa < 0.40$).

When the data were analyzed on the basis of farms, 15 (17.2%) farms were found to be negative for both PRRS virus strains. Of the 72 farms that were positive, 7 (9.7%) were positive for antibodies to Lelystad virus only, 14 (19.4%) were positive for VR-2332 antibody only, and 51 (70.8%) were positive for antibodies against both strains. These results support previous observations⁹ in which antigenic differences were found among European and American isolates of PRRS virus. The results of the present study are also in agreement with those of a previous study from our laboratory in which phenotypic differences were found among PRRS virus strains, e.g., absence or delayed onset of cytopathic effects in infected cells and the failure of the virus to grow in 1 or the other cell system?

Although many animals had antibody to either the Lelystad or the VR-2332 strain, there were several sera that had antibodies to both strains. This finding may be the result of coinfection of an animal with both strains of the virus or of antigenic cross-reaction between the 2 strains as seen later in infection. A higher prevalence of antibodies to the VR-2332 strain was found in this study, as indicated by the following observations: 1) 210 (43.8%) samples had antibodies to VR-2332 only, whereas 96 (20.1%) samples were positive for antibody to Lelystad virus only (Table 1), and 2) of the 173 samples that had antibodies against both viruses, 122 samples had higher titers to VR-2332 than to Lelystad virus versus 10 samples that had higher titers to Lelystad virus than to VR-2332 virus. However, evidence for the American swine herds being affected with a Lelystad-like strain was also found, which is disconcerting because such animals cannot be detected by the currently used diagnostic tests, especially early in the course of infection. Thus, in the present study, virus infection in approximately 20%

(96/479) of the samples and 10% (7/72) of the farms was missed when using only the VR-2332 strain as indicator for the IFA test. Three farms from Minnesota, 1 from Kansas, 2 from Alabama, and 1 from Colorado were positive for antibodies to the Lelystad strain only (Table 1). Farms from Iowa, Illinois, Indiana, Michigan, Nebraska, and North Carolina had individual animals that were seropositive for both Lelystad and VR-2332 viruses, with some animals being positive for only 1 strain. Whether this finding is due to a mixed infection or to the occurrence of cross-reactions during the later stages of infection is unknown.

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Sources and manufacturers

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- b. Robert Morrison, University of Minnesota, St. Paul, MN.
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- d. Cappel Organon Teknika, Durham, NC.

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Competitive ELISA for serodiagnosis of bluetongue: a refinement

A. Afshar, G. C. Dulac, C. Dubuc, J. E. Pearson, G. A. Gustafson

Several group specific monoclonal antibody (MAb)-based enzyme-linked immunosorbent assays (ELISAs) have been developed for the detection of antibodies to the group-specific antigens of bluetongue virus (BTV). In 1984, a blocking (B) ELISA was described in which the immobilized BTV antigen was first reacted with a test serum and then with a BTV group-specific MAb (3.17.A3) from Pirbright Laboratory (PL).⁵ In 1987, a competitive (C) ELISA was developed in which the test serum and the same MAb (3.17.A3) were reacted simultaneously to compete for BTV antigen coated on polystyrene plates.³ The C.ELISA was more sensitive and specific than the agar-gel immunodiffusion, the modified complement fixation, the plaque neutralization, and the in-

direct ELISA tests.⁴ Several other laboratories have adopted a competitive or a blocking format, using PL MAb 3.17.A3 or other MAbs, and have clearly demonstrated the advantages of these assays for the detection of group-specific antibodies to BTV.^{8-10,13} In a recent study on several coded panels of bovine sera, both C.ELISA and B.ELISA demonstrated comparable performance. However, the advantage of the C.ELISA over the B.ELISA format is that it requires less time to perform.¹

For the past 4 years, Agriculture Canada's Animal Diseases Research Institute, Nepean (ADRI), and USDA's National Veterinary Services Laboratories, Ames (NVSL), have extensively applied the C.ELISA, using the PL MAb 3.17.A3, for serologic surveys and for certification of animals prior to exportation and importation. The assay is also used for monitoring cattle at artificial insemination (AI) centers and for sentinel cattle herds used in the Okanagan Valley as an important improvement of bluetongue (BT) surveillance in Canada.⁷ Initially, based on the testing of serum samples collected from calves and sheep experimentally infected with

From the Animal Diseases Research Institute, Agriculture Canada, PO Box 11300, Station H, Nepean ON K2H 8P9, Canada (Afshar, Dulac, Dubuc). and the National Veterinary Services Laboratories, USDA-APHIS, PO Box 844, Ames, IA 50010 (Pearson, Gustafson).

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