Ontario

Lelystad-like strain of porcine reproductive and respiratory syndrome virus (PRRSV) identified in Canadian swine

In May 1999, 350 pigs were imported to Canada from The Netherlands to be used as breeding stock. These animals were cesarian-derived and raised in isolation until they were 2 to 3 wk of age, at which time they were shipped by air to Canada. The animals were kept in a federally controlled isolation barn for a 3-month quarantine period and then moved to a farm-controlled isolation unit near the breeding stock farm. Subsequent to the move, the group experienced an outbreak of coughing. In August 1999, tonsillar biopsies were taken from 26 of the animals and blood was taken from 30 of the animals.

The tonsillar biopsies were tested at the Veterinary Services Branch of Manitoba Agriculture for the presence of porcine reproductive and respiratory syndrome virus (PRRSV) by using nested PCR technology. Two different nested PCR assays were used that amplified 300 base pair regions in open reading frame (ORF) 7. One assay used primers derived from sequences specific for Lelystad-like PRRSV strains; the other used primers derived from sequences specific for North American strains. The PCR assay was performed by using methods, essentially, as previously described (1,2). By using PCR, 13 of the tonsillar biopsies were found to be positive for PRRSV antigen. Eleven of the 13 samples had over 99% homology with Lelystad-like PRRSV, as determined by DNA sequencing. Results of DNA sequencing from the other 2 positive tonsillar biopsies were found to be similar to North American strains, suggesting a dual infection of the herd with both Lelystad-like and North American strains of PRRSV. The 30 sera were tested for antibody to PRRSV at the Animal Health Laboratory, University of Guelph. By using the IDEXX PRRSV ELISA (IDEXX Laboratories, Westbrook, Maine, USA), 26 of the 30 blood samples were positive for antibody to PRRSV (range of S/P ratios: 0.582 to 2.332) and 4 were negative for antibody (< 0.4).

In December 1999, 104 of the 350 imported animals were tested again with the ELISA for antibody to PRRSV. None of these animals had been included in the initial group of 30 pigs that had been tested in August. Two animals were antibody negative, 86 had positive S/P ratios between 0.417 and 2.451, and 16 had positive S/P ratios ranging from 2.510 to 3.740. Sera from 20 pigs that were positive for antibody to PRRSV by using the ELISA, with a range of S/P ratios from 0.804 to 2.806, were selected for further testing. These samples were forwarded to the National Veterinary Services Laboratories (NVSL) in Ames, Iowa, USA, for testing in 2 distinct differential indirect immunofluorescence assays, one using the NVSL North American strain of PRRSV (similar to VR-2332) to detect antibody to North American strains of PRRSV, and a second using the Lelystad PPRSV to detect antibody to Lelystad-like strains of PRRSV. Of the 20 sera tested in both assays, 19 were positive for antibody for only the Lelystad strain of PRRSV, with titers ranging from 1/80 to 1/1280. One sample, with an antibody titer of > 1/1280 to the Lelystad PRRSV, also had an antibody titer of 1:320 to the North American strain of PRRSV. These animals have remained in the breeding stock herd in Ontario.

To the best of the authors' knowledge, this is the first definitive report of Lelystad- like PRRSV infection detected by PCR in Canadian swine; it is also the first reported case of dual infection with both North American and Lelystad-like PRRSV present in the same swine herd. Lelystad-like strains of PRRSV may not have been identified earlier because diagnostic tests used in North America have not been designed for the specific identification of Lelystad-like strains, even though there has been serologic evidence of Lelystad-like PRRSV in North America since 1993 (3) using similar indirect immunofluorescence tests. For example, the IDEXX PRRSV ELISA antibody test does not differentiate between PRRSV strains, because the antigen-coated microtiter plates include both North American and Lelystad-like antigens. Also, most laboratories in North America currently use PCR tests including ORF7 primers that are specific for identification of North American strains of the PRRSV and do not include specific ORF7 primers needed to identify Lelystad-like strains of PRRSV. Other laboratories use ORF7 primers designed to detect both the North American and Lelystad-like strains, but may not have been able subsequently to differentiate between these strains by using ORF5 primers and restriction fragment length polymorphism, as described by Wesley (4). Lastly, North American strains of PRRSV grow relatively well in MARC-145 cell lines. However, Lelystad-like strains grow more readily in primary alveolar macrophages. Many laboratories in North America use only MARC-145 cells because of the relative difficulty of establishing primary alveolar macrophages for cell culture for routine virus isolation.

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Québec

Canine leptospirosis: serology

In recent years, acute renal failure associated with leptospiral serovars other than *icterohaemorrhagiae* and *canicola* has appeared as an emerging syndrome in dogs in Quebec (1), Ontario (2), and northeastern United States (3). In 1999, sera from 19 dogs affected with acute renal failure, or suspected of having a leptospiral infection, underwent serological testing for leptospirosis, using the microscopic agglutination test (MAT) with antigens of serovars *pomona, grippotyphosa, hardjo, icterohaemorrhagiae,* and *bratislava.* Tests were performed at the laboratory of the Direction de la qualité des aliments et santé animale du Ministère de l'agriculture, des pêcheries et de l'alimentation du Québec in Ste-Foy. No convalescent sera were tested from these animals.

One of 19 sera was negative for all 5 leptospiral serovars, and 3 had a titer of less than 1:100 against only 1 serovar of *Leptospira interrogans.* Of these 3 sera, 2 reacted with serovar *bratislava* and 1 with serovar *grippotyphosa.* **The distribution of titers for the other 15 sera is presented in Table 1. Results in Table 1 indicate that titers were predominantly directed against serovar *grippotyphosa* and/or *pomona* (87%). Among sera reacting mainly against serovar *grippotyphosa,* 6 had titers ≥ 1:6400 and 5 were ≥ 1:12 800.** Thirteen of the 15 sera in Table 1 had titers against serovar *bratislava,* but except for 1 case (#12), the highest titers were against other serovars. In case #12, the titer against serovar *bratislava* exceeded that against *grippotyphosa* by only 1 dilution, which is not considered a significant difference. Two cases showed a titer of 1:1600 against serovar *hardjo,* but in both cases, the titer against serovar *grippotyphosa* was at least 1:51 200. Finally, in all cases, only weak reactions against serovar *icterohaemorrhagiae* were detected.

The involvement of serovars *grippotyphosa* and *pomona* as the major cause of renal failure in dogs has been reported by other authors (2,3). Those authors associated the infection in dogs with the presence in urban areas of wild carrier animals, such as raccoons and skunks (1,2). The determination from single

Table 1. Distribution of titers against 5 different *Leptospira interrogans* serovars, by using the microscopic agglutination test, from sera of 15 dogs affected with acute renal failure

Serum no.	*pomona*	*grippotyphosa*	*hardjo*	*icterohaemorrhagiae*	*bratislava*
1	6400	800	200	< 100	1600
2	12 800	3200	< 100	100	3200
3	400	51 200	1600	100	400
4	1600	25 600	—	—	6400
5	—	400	400	—	—
6	—	800	—	—	—
7	1600	12 800	—	100	3200
8	1600	25 600	—	—	6400
9	1600	800	—	—	800
10	800	200	—	100	200
11	200	51 200	1600	—	1600
12	400	800	800	—	1600
13	1600	200	—	400	100
14	400	3200	—	—	1600
15	200	6400	—	—	200