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Comparison of two commercial enzyme-linked immunosorbent assays for the diagnosis of *Porcine reproductive and respiratory syndrome virus* infection

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Abstract. Early diagnosis of *Porcine reproductive and respiratory syndrome virus* (PRRSV) is critically important for control of the disease. Two new commercially available enzyme-linked immunosorbent assays (ELISAs) based on different methodologies have been developed. In the present report, the 2 ELISAs were compared using blood samples from experimentally and naturally infected pigs. One of the 2 ELISAs was shown to be more sensitive than the other. The higher sensitivity of one of the ELISAs could pose a problem in PRRS diagnosis in endemic farms, because it can detect maternally derived antibodies for a longer time, overlapping with the detection of antibodies developed after PRRSV infection. However, the ELISA with higher sensitivity could be suitable for early detection of PRRSV antibodies in individual pigs, especially in PRRS-free herds.[AQ: 2]

Key words: Early diagnosis; enzyme-linked immunosorbent assay; porcine reproductive and respiratory syndrome; pigs..

To date, both inactivated and attenuated vaccines against *Porcine reproductive and respiratory syndrome virus* (PRRSV; order *Nidovirales*, family *Arteriviridae*, genus *Arterivirus*) are available, but control and eradication of the disease are far from being achieved.[2] Early and accurate diagnosis is important for establishment of a control program. At present, the earliest way to detect a positive animal is by reverse transcription polymerase chain reaction (RT-PCR). If detection of antibodies was feasible at very early times postinfection, the cost of early diagnosis could be substantially lowered.

The most abundant viral protein in PRRSV is the nucleocapsid (N) protein that is highly immunogenic and induces early antibody responses in pigs.[3] This protein has been used extensively in the development of commercial enzyme-linked immunosorbent assays (ELISAs) for PRRSV diagnosis. Recently, 2 PRRSV ELISAs based on different methodologies (double-recognition ELISA and indirect ELISA) have been developed for the detection of antibodies against N protein. The principle of the double-recognition ELISA is based on simultaneous binding of an antibody sandwiched between precoated and enzyme-conjugated antigens.[4] The double-recognition ELISA used in the present study (E1,[a] hereafter) was developed with recombinant N protein from genotype I PRRSV serving as both the coating antigen and, in horseradish peroxidase (HRP)–conjugated form, the signal. Briefly, after coating with recombinant N protein, plates were washed 3 times and blocked with bovine serum albumin diluted at 1% in phosphate buffered saline. Then, 20 µl of sera were diluted 1:5 in 80 µl of the HRP-conjugated N protein and added in a one-step incubation. Then, the reaction was revealed by the addition of tetramethyl benzidine substrate and stopped with 0.5 M sulfuric acid. The indirect ELISA (E2,[b] hereafter) was designed to detect PRRSV antibodies using a recombinant protein as the coating antigen, and an anti-pig immunoglobulin (Ig)G-HRP conjugate in a second step. The diagnostic specificity of E1 is 99% (Ranz A, et al.: 2010, 4th Annual Meeting EPIZONE, 7–10 June 2010, Saint Malo, France), and the diagnostic specificity of E2 was 99.9% (Leathers V, et al.: 2009, International PRRS Symposium, 4–5 December 2009, Chicago, IL). The present report describes the comparison of these new commercially available ELISAs for detection of PRRSV antibodies in pigs experimentally and naturally infected with genotype I strains.

Four-week-old piglets (n = 36) obtained from a high-health farm historically free of PRRSV and reconfirmed to be free of PRRSV by ELISA and RT-PCR were experimentally

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