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Short Communication

Comparison of the immunoperoxidase monolayer assay and three commercial ELISAs for detection of antibodies against porcine circovirus type 2

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ABSTRACT

The aim of this study was to compare and correlate antibody titres against porcine circovirus type 2 (PCV2) in porcine sera ($n = 1270$) obtained by immunoperoxidase monolayer assay (IPMA) with the results of three commercial ELISAs (designated E1, E2 and E3). The correlation between IPMA and ELISA results was excellent ($r^2 \geq 0.90$). Compared to IPMA, E2 had the highest sensitivity (93.0%), followed by E3 (90.1%) and E1 (85.0%); the specificity was 100% for all tests. All three commercial ELISAs had predictive values similar to those of IPMA and could be used to monitor antibody responses against PCV2 infection and/or vaccination.

The immunoperoxidase monolayer assay (IPMA) is used widely for detection of antibodies against porcine circovirus type 2 (PCV2) (Opriessnig et al., 2007; Fort et al., 2009; Fraile et al., 2012a, 2012b). Neutralising antibodies (NAs) are the main antibodies responsible for protection and clearance of PCV2 infection (Meerts et al., 2005; Fort et al., 2007). Since there is a positive correlation between IPMA titres and titres of NAs (Fort et al., 2008, 2009), IPMA titres might be considered to be an indirect measure of NAs. Furthermore, high levels of maternally derived antibodies (IPMA titres ≥10 $\log_2$) appear to interfere with the development of humoral immunity after vaccination, while IPMA levels <8 $\log_2$ do not have this effect (Fort et al., 2009; Fraile et al., 2012a, 2012b).

IPMA is a relatively complex technique for routine diagnostic use, since it depends on the availability of virus infected cell cultures, requires a high level of technical expertise and is relatively slow for screening large numbers of sera. For these reasons, replacement of IPMA by automated serological tests with an objective end-point reading system, such as ELISAs, is desirable. The aim of this study was to compare and correlate the antibody titres determined by IPMA with the results of three commercial ELISAs and to determine if ELISA results can be useful to infer IPMA titres.

Sera used in this study ($n = 1248$) came from a previous study performed on a commercial farm with a diagnosis of PCV2-systemic disease (Fraile et al., 2012b). One week before mating, 57 sows were randomly divided into two groups: (1) vaccinated sows (V; $n = 26$) receiving an intramuscular dose of Porcilis PCV (Intervet International BV); and (2) unvaccinated sows (not vaccinated, NV, $n = 31$) receiving phosphate buffered saline (PBS) as a placebo. At 4 weeks of age, 208 healthy piglets from these sows were divided into two groups; 106 piglets were vaccinated with Porcilis PCV and 102 piglets received only PBS: (1) NV piglets from NV sows (NV-NV, $n = 50$); (2) V piglets from NV sows (NV-V, $n = 52$); (3) NV piglets from V sows (V-NV, $n = 52$); and (4) V piglets from V sows (V-V, $n = 54$). Blood samples were collected from piglets at 4, 12, 16, 21 and 26 weeks of age. In addition, 22 sera from 7- to 10-day-old Caesarean-derived, colostrum-deprived (CDCD) piglets were included in the study as negative controls. The study was approved by the Ethical and Animal Welfare Committee of the Universitat Autònoma of Barcelona (approval number 665M2; date of approval 24 October 2013).

All serum samples ($n = 1270$) were analysed for anti-PCV2 antibodies by IPMA and three commercial ELISAs: (1) SERELISA PCV2 Ab Mono Blocking (Synbiotics; E1); (2) Ingezim Circo IgG 11. PCV.K1 (Ingenasa; E2); and (3) PCV2 ELISA SK105 (Biochek; E3). Serum samples were analysed by IPMA in four-fold dilutions from 1:20 to 1:20,480 (Rodríguez-Arrioja et al., 2000). Results were expressed as $\log_2$ of the inverse of titre values. All samples with ≥4.32 $\log_2$ titre were considered to be positive. ELISAs were performed according to the manufacturers' instructions and sera were analysed at 1:1000, 1:200 and 1:50 dilutions by E1, E2 and E3, respectively.

ELISA values were expressed as antibody titres according to the mathematical formula provided by each manufacturer and the corresponding plate specific cut-off values were applied to convert the ELISA results into categorical data. Results for E1 (blocking ELISA)

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