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Application of Oral Fluid Sample to Monitor *Porcine circovirus-2* Infection in Pig Farms

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Abstract : *Porcine circovirus-2* (PCV2) has been implicated in many clinical diseases/syndromes that are now referred to as PCV-associated diseases (PCVAD). Due to significant economic losses caused by PCVAD, many swine operations have launched extensive monitoring programs for PCV2. Traditional serum sampling is, however, rather expensive and laborious, hampering effective large scale pathogen surveillance. A field-based longitudinal study was conducted to assess the utility of pen-based oral fluid sample as an alternative to serum for herd PCV2 testing. Six pens (25 pigs/pen) at each of 3 different sites were used in the study. One oral fluid and 5 random serum samples per pen were collected at 3, 5, 8, 12, and 16 weeks of age, and the sera were pooled by pen for testing. All samples were tested for PCV2 by real-time PCR and for antibodies by indirect fluorescent antibody test (for both anti-PCV2 IgG and IgA) and 3 ELISA assays (blocking ELISA, indirect ELISA, and IgG/IgM sandwich ELISA). PCV2 DNA was detected in oral fluid samples sporadically until 8 weeks and in all pens at 16 weeks. PCV2-specific IgG was detected in oral fluid samples at 3 weeks and persisted until 5 to 8 weeks in all sites. Anti-PCV2 IgG and IgA were detectable in oral fluid samples collected at 16 weeks from all of the pens at 1 site. The detection of PCV2 and anti-PCV2 antibody in oral fluid samples correlated positively with results on pooled sera, suggesting that oral fluids can be a cost-effective alternative to serum for herd monitoring of PCV2 infection.

Key words **:** antibody detection, enzyme-linked immunosorbent assay, indirect fluorescent antibody test, oral fluids, *Porcine circovirus-2.*

Introduction

Porcine circovirus-2 (PCV2; family *Circoviridae*, genus *Circovirus*) is a nonenveloped virus with single-stranded circular DNA (2). Infection with PCV2 has been implicated in many clinical diseases/syndromes, which are now commonly referred to as PCV-associated diseases (PCVAD) (14), such as postweaning multisystemic wasting syndrome (PMWS) (13,23), porcine dermatitis and nephropathy syndrome (PDNS) (31,35), proliferative or ulcerative enteritis, abortion, and pneumonia (1,14). Epidemiological data has demonstrated that PCV2 infection is widespread in the pig population but only a few of the infected pigs manifest PCVAD (34). In addition, the virus has been detected in pigs from both PMWS-affected and -nonaffected farms (6,19,29). Although the detection of PCV2 or virus-specific antibodies in clinical samples without clinical and pathological assessment is not considered to be diagnostic of PCVAD (14,32), several studies have demonstrated that the level of PCV2 in serum and tissues tends to be significantly higher in clinically affected pigs than in healthy pigs (4,20,22).

Recently, a severe form of PCVAD caused significant economic losses to the swine industry (8,10,14,33,37). In response, commercial PCV2 vaccines have been made available to swine practitioners and producers (16). Many swine operations have launched an extensive and proactive monitoring program for early detection of PCV2 infection and/or to determine the time for prophylaxis.

Traditionally, herd monitoring uses sera for testing. However, serum sampling is rather expensive and laborious and often hampers large scale pathogen surveillance. Oral fluid samples, which are composed of saliva, crevicular fluid (i.e., fluid from the crevice between the teeth and gums), membrane transudates, and food residues, have been suggested as an alternative sample to serum for detecting agents and/or pathogen-specific antibody (9,15,21). Oral fluid samples enriched in crevicular fluid and membrane transudates contain a relatively high level of immunoglobulin G (IgG) and could reflect the level of antibodies in serum (7,12,15). Since oral fluid (or saliva) collection is rapid, noninvasive, and less objectionable to patients, it has been successfully applied to the detection of antibody against *Human immunodeficiency virus* (HIV), *Hepatitis C virus* (HCV), and *Human papillomavirus* (HPV) (7,15). A recent study demonstrated the usefulness of oral fluid samples for detection of *Porcine reproductive and respiratory syndrome virus* (PRRSV) and anti-PRRSV antibody (28). The present report describes a field-based longitudinal cohort study

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to assess the utility of pen-based oral fluid samples as an alternative to serum for herd PCV2 testing.

Materials and Methods

Target population and sampling

On 3 finishing sites that were historically known through laboratory diagnostic testing to be exposed to PCV2, pens of approximately 20-30 pigs each were monitored for PCV2 infection using oral fluid and serum samples. Six pens at each of the 3 sites were selected to be monitored. Samples (serum and oral fluid) were collected when pigs entered the facilities at 3 weeks of age, and subsequently at 5, 8, 12, and 16 weeks of age.

At each sampling time, blood samples were collected via venipuncture from 5 randomly selected pigs per pen. Serum was harvested from each clotted blood sample after centrifugation at 1000 × *g* for 10 min and pooled by pen for testing. Oral fluid samples were collected using a length of 3-strand twisted cotton material suspended in an accessible location in the pen. After being chewed on by the pigs for approximately 30 min, oral fluid was extracted and collected as previously reported (28). The samples were centrifuged at 1000 × *g* for 10 min to remove food debris before storage at −80°C. A total of 90 samples (18 pens × 5 sampling points) of each type (i.e., oral fluid or pooled serum) were collected for the study.

Indirect fluorescent antibody test (IFA)

Porcine circovirus-2 and control cell antigens for IFA were prepared in 96-well plates using PK (porcine kidney)-15 cells as previously described (27). Pooled serum and oral fluid samples were diluted at 1:5 in 0.01 M of phosphate buffered saline (PBS; pH 7.2) containing 0.05% Tween20 (PBST) for initial testing. Any positive samples were then further diluted in PBST using a serial 2-fold dilution technique for determination of antibody titer. Known PCV2-positive and -negative serum samples were diluted at 1:20 and served as the controls for the assay.

For testing, the plates were first washed with PBST. Fifty µl of each diluted sample or control sera were added to a well of the plate. The plates were incubated at 37°C for 1 hr and washed 3 times with 150 µl/well of PBST. Fifty µl of goat anti-swine IgG (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA), IgA (Bethyl Laboratories Inc., Montgomery, TX, USA), or IgM antibody (Kirkegaard & Perry Laboratories) conjugated with fluorescein isothiocyanate (FITC) was added to each well and the plates were incubated at 37°C for another hour. The plates were then washed 3 times with PBST and allowed to air dry in the dark. The plates were read qualitatively under a fluorescent microscope. Indirect fluorescent antibody titer was determined as the reciprocal of the highest dilution in which specific fluorescence was observed.

Enzyme-linked immunosorbent assays

Three different ELISA formats were used in the current study. An indirect ELISA (hereafter, ISU ELISA), which was designed to detect IgG antibody specific for the capsid protein [ORF (open reading frame) 2 product] of PCV2 (24), was performed on serum at 1:100 dilution and on oral fluid samples at 1:100 and 1:5 dilutions. A commercial blocking ELISA (SERELISA® PCV2 Ab Mono Blocking, Synbiotics Corp., Lyon, France), which has been reported to detect antibodies of all isotypes (25,36), was conducted on serum at 1:10 dilution as per the manufacturer's recommended protocol, and on oral fluid samples at 1:5 and 1:10 dilutions. A commercial sandwich ELISA (INGEZIM Circovirus IgG®/IgM®, INGENASA, Madrid, Spain), which is labeled for detection of both IgM and IgG specific for PCV2 in a differential manner (25), was utilized to test serum at 1:100 dilution by following the recommended manufacturer's procedure. The INGEZIM ELISA was not performed on oral fluid samples because very poor performance was observed when it was used on serum samples collected for the current study.

Quantitative real-time polymerase chain reaction

Active PCV2 infection in animals tested was determined based on the detection of viremia by a fluorogenic polymerase chain reaction (i.e., real-time PCR) using TaqMan protocols (26). The presence/circulation of PCV2 in a population was determined based on the detection of PCV2 in oral fluid samples by the same real-time PCR. Polymerase chain reaction primers (Integrated DNA Technologies Inc., Coralville, IA, USA) and probe (TAMRA™ Probe, Applied Biosystems, Foster City, CA, USA) with 5' reporter 6-carboxyfluorescein (FAM) and a 3' 6-carboxytetramethylrhodamine (6-TAMRA) quencher [forward primer (P1570): 5'-TGGCCCGCAGTATTCTGATT-3'; reverse primer (P1642): 5'-CAGCTGGGACAGCAGTTGAG-3'; and probe (P1591): 5'-6FAM-CCAGCAATCAGACCCCGT-TGGAATG-TAMRA-3'] were designed to detect complementary sequences in ORF1 of PCV2 and synthesized by commercial vendors.

For assay, viral DNA was extracted from 175 µl of each sample using a commercial isolation kit (MagMax™ Viral RNA Isolation Kit, Ambion, Austin, TX USA) with modification. Briefly, 175 µl of sample was added to 235 µl of lysis buffer and incubated for 5 min with shacking. After the incubation, the mixture were centrifuged at 4000 × *g* for 10 min and 115 µl of supernatant was transferred to a 96-well deep-well microplate (VWR, West Chester, PA, USA) containing 65 µl of 100% isopropanol and 20 µl of paramagnetic beads. The deep-well microplate was placed in KingFisher magnetic particle processor (Thermo Scientific, Waltham, MA, USA) for automated extraction process as described in the manufacturer's manual. After the automated process, viral DNA was extracted in 50 µl of elution buffer. Real-time PCR was then carried out with TaqMan® Fast Universal PCR Master Mix (Applied Biosystems) in the 25-µl reaction volume using 5 µl of extracted template. Primers were added at a final concentration of 20 µmol each; the probe was at a final concentration of 25 µmol. The PCR amplification was performed on the ABI 7900HT Sequence Detection System (Applied Biosystems). Cycling conditions were as follows: activation step at 95°C for

20 sec, and 40 cycles of 3 sec at 94°C and 30 sec at 60°C. A known PCV2 preparation was used as the positive control. Samples with a threshold cycle (Ct) ≤ 35 cycles were considered positive. The amount of PCV2 in each sample was calculated by converting Ct value to virus titer [fluorescence-forming focus unit (FFU) per 1 ml] using the standard curve established with viruses of known titers.

Sequencing

The target gene (ORF2) was amplified using the QIAGEN® One-Step PCR kit (Qiagen Inc., Valencia, CA, USA). PCR products were purified using the QIAquick® PCR purification kit (Qiagen Inc.) and submitted for sequencing. Primers for PCR and sequencing were as follows: ORF2 forward (PCV2SEQF2): 5'-CCGCAGTATTCTGATTACCAGCAAT-3'; and ORF2 reverse (PCV2SEQR2): 5'-CTGCCGCTGCCGAAGTGC-3'.

Data analysis

Nucleic acid sequences of ORF2 were aligned and analyzed using Lasergene® MegAlign software (DNASTAR Inc., Madison, WI, USA). All other data were statistically analyzed with JMP (SAS institute Inc., Cary, NC, USA). The repeated measurements of the level of antibody and viral genome in serum and oral fluid samples were analyzed with repeated measures ANOVA. In addition, the performance of the different serologic tests was compared by calculating % agreement with IFA results and kappa (κ) value as follows:

% agreement = [agreed pos + neg/total tests (n = 90)] × 100,

$\kappa = [Pr(a) - Pr(e)]/[1 - Pr(e)]$,

where $Pr(a)$ is the relative observed agreement between tests, and $Pr(e)$ is the probability that agreement is due to chance. If the tests are in complete agreement, then $\kappa = 1$. If there is no agreement between the tests, then $\kappa = 0$. The interpretation of κ value was based on a previous guide (18): poor agreement ($\kappa = 0.00$); slight ($0.01 < \kappa < 0.20$); fair ($0.21 < \kappa < 0.40$); moderate ($0.41 < \kappa < 0.60$); substantial ($0.61 < \kappa < 0.80$); and almost perfect ($0.81 < \kappa < 1.00$).

Results

Detection of PCV2 viremia

The presence of PCV2 viremic pigs with increasing virus titers was detected from all 3 sites after 8 weeks of age (Table 1, Fig 1A), suggesting that active PCV2 infection was established in the animals at some point during the observation period. At 16 weeks of age, the level of viremia ranged from 2×10^2 to 8×10^6 FFU equivalent/ml when pooled serum samples were tested. Based on the sequencing data, all of the viruses circulating in all 3 sites were PCV2 of North American genotype (PCV2a or PCV2 genotype 2), and their sequence homologies ranged from 99.6% to 100%.

Detection of PCV2 in oral fluid samples

PCV2 DNA was detected at a level lower than $1 \times 10^{1.5}$ FFU equivalent/ml on average in oral fluid samples from 19 pens

Table 1. Detection of *Porcine circovirus-2* in pooled sera and oral fluid samples over time after placement of animals in 1 of 3 different sites*

Age (weeks)	Site 1		Site 2		Site 3	
	Serum	Oral fluids	Serum	Oral fluids	Serum	Oral fluids
3	0/6	2/6	0/6	0/6	0/6	0/6
5	1/6	3/6	0/6	1/6	0/6	0/6
8	0/6	3/6	0/6	2/6	0/6	0/6
12	5/6	2/6	1/6	5/6	0/6	1/6
16	6/6	6/6	6/6	6/6	6/6	6/6

*Number of positive pens/total number of pens tested (n = 6). At each sampling time (age), the samples were collected from pigs in 6 pens per site.

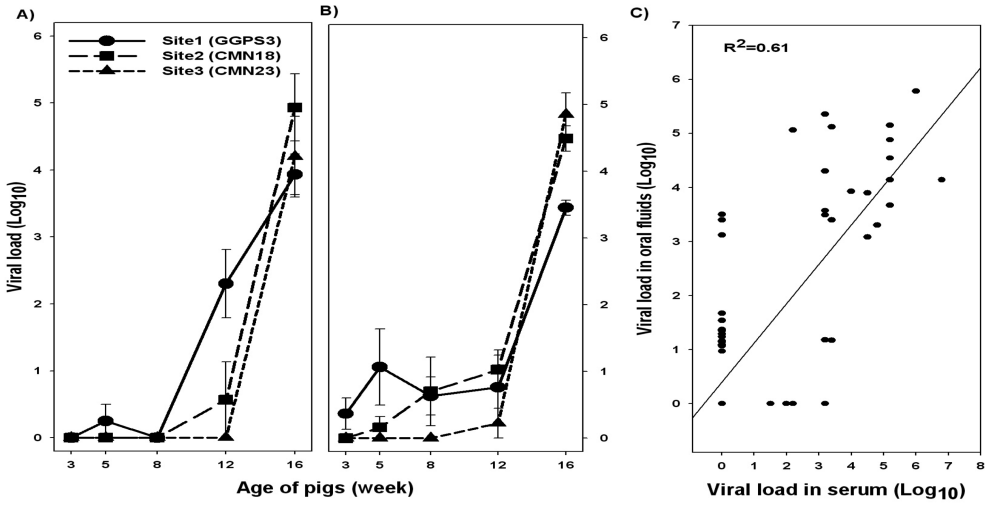

Fig 1. The level [fluorescence-forming focus unit (FFU) equivalent/ml] of *Porcine circovirus-2* in serum (A) and oral fluid samples (B) over time as determined by a quantitative real-time PCR and the correlation between viral loads in serum and oral fluid samples (C).

(10 in site 1, 8 in site 2, and 1 in site 3) until 12 weeks of age when PCV2 level in oral fluid samples was increased to 3×10^3 FFU equivalent/ml (Table 1, Fig 1B). Similar to the PCV2 viremia pattern, the level of virus in oral fluid samples collected from all sites at 16 weeks of age was greatly increased ($1 \times 10^3 - 8 \times 10^5$). Overall, a significant correlation ($r^2 = 0.61$, $p < 0.0001$) between viral load in oral fluid samples and that in serum was observed (Fig 1C).

Detection of PCV2-specific antibody in serum samples

Serologic profiles of pigs in pens and sites for PCV2 exposure as determined by 4 different assays (IFA, ISU ELISA, SERELISA, and INGEZIM ELISA) over time are summarized in Table 2 and Fig 2A, C, and E. Neither IgA nor IgM was detected by IFA in any of the pooled sera tested. All pens in all of the 3 sites were seropositive for PCV2 specific IgG until 5 weeks of age by IFA and until 8 weeks of age by ISU ELISA. Results of SERELISA during the same period (i.e., up to 8 weeks of age) were similar to those of IFA and ISU ELISA, but the number of seropositive pens detected by SERELISA was less than that detected by the other 2 tests. None of the study pens, with the exception of 1 pen each in sites 1 and 2, was seropositive for PCV2 at 12 weeks by IFA and SERELISA. However, all pens in site 1 and 3-5 pens in sites 2 and 3 became

Table 2. Detection of *Porcine circovirus-2*–specific antibodies in pooled sera by various serological assays*

Age (weeks)	Site 1					Site 2					Site 3				
	IFA (IgG)	SERE-LISA	ISU ELISA (IgG)	INGEZIM ELISA		IFA (IgG)	SERE-LISA	ISU ELISA (IgG)	INGEZIM ELISA		IFA (IgG)	SERE-LISA	ISU ELISA (IgG)	INGEZIM ELISA	
				IgG	IgM				IgG	IgM				IgG	IgM
3	6/6	2/6	6/6	0/6	6/6	6/6	6/6	6/6	0/6	5/6	6/6	5/6	6/6	1/6	6/6
5	6/6	3/6	6/6	0/6	6/6	6/6	3/6	6/6	0/6	2/6	6/6	2/6	6/6	0/6	6/6
8	0/6	2/6	6/6	0/6	6/6	0/6	1/6	6/6	0/6	0/6	6/6	0/6	6/6	0/6	6/6
12	0/6	0/6	1/6	0/6	6/6	0/6	0/6	1/6	0/6	0/6	0/6	0/6	0/6	0/6	6/6
16	6/6	0/6	6/6	5/6	6/6	5/6	0/6	3/6	0/6	0/6	4/6	0/6	4/6	0/6	6/6

*Number of positive pens/total number of pens tested (n = 6). At each sampling time (age), sera were collected from pigs in 6 pens per site. Each serum was tested after diluted as recommended by the manufacturer or the developer and represents a pool of 5 sera per pen. IFA = indirect fluorescent antibody test; ISU ELISA = Iowa State University-developed enzyme-linked immunosorbent assay.

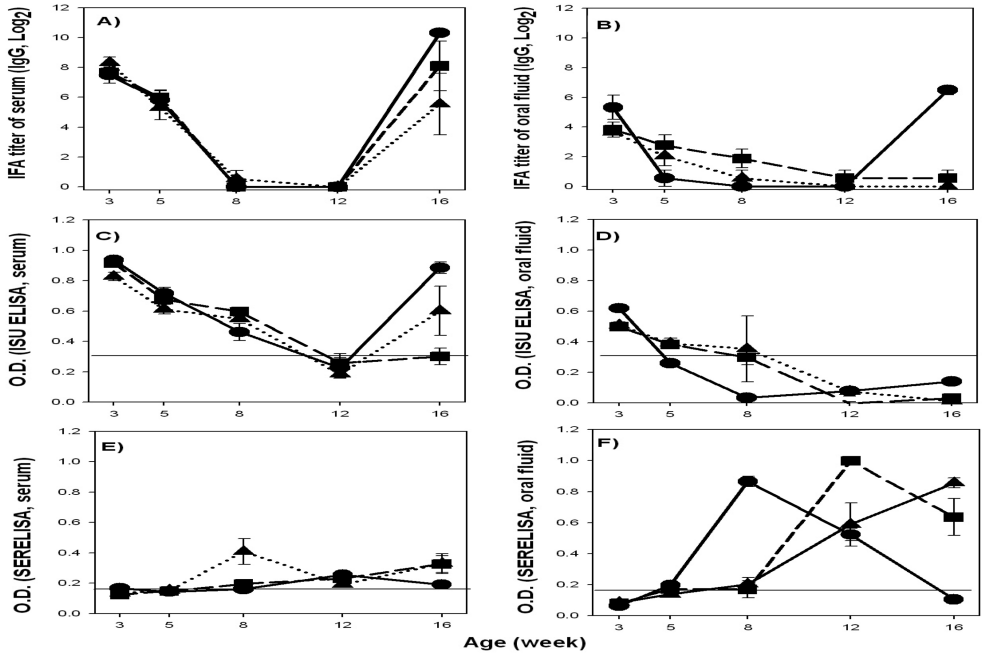

Fig 2. Anti-*Porcine circovirus-2* IgG antibody responses in serum (A, C, E) and oral fluid samples (B, D, F) as determined by indirect fluorescent antibody test (A, B), ISU ELISA (C, D), and SERELISA (E, F). While serum samples were diluted 1:100 or 1:10 for ISU ELISA and SERELISA, respectively, as recommended by the manufacturer or developer, oral fluid samples were diluted 1:5 for testing. Horizontal line in each panel indicates the cut-off values for each test.

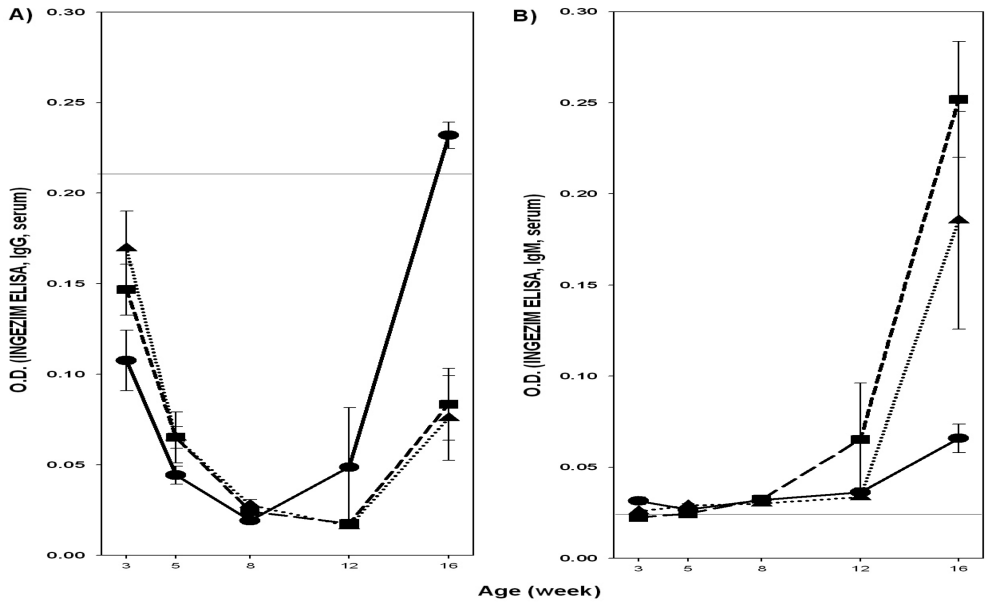

Fig 3. IgG (A) or IgM (B) antibody response determined in serum by INGEZIM ELISA. Horizontal line in each panel indicates the cut-off values for each test.

seropositive for PCV2 at 16 weeks by IFA and ISU ELISA, whereas no site was seropositive for PCV2 by SERELISA, suggesting different levels of sensitivity among the tests.

In contrast, INGEZIM ELISA (designed to detect both IgG and IgM in a differential manner) was inefficient for detecting PCV2- specific IgG as compared to the other serologic assays employed in this study since only 6 of the 90 samples were determined to be positive for anti-PCV2 IgG by this assay (Table 2, Fig 3A). The assay determined all except 6 pens in site 2 to be positive for IgM specific for PCV2 (Table 2). A significantly higher level of PCV2-specific IgM antibody was detected in all sites after 12 weeks of age ($p < 0.0001$), implying that there was a recent infection by PCV2 (Fig 3B).

Comparison of assay performance on serum samples

The performance of the 3 ELISAs on pooled serum samples was compared to that of IFA (Table 3). IFA and SERELISA showed 62% agreement in test results ($\kappa = 0.296$), which indicated a fair level of agreement. Much less agreement in test results (49%) was observed between ISU ELISA and SERELISA, which could be attributed to the higher sensitivity of ISU ELISA. IFA and ISU ELISA represented a 76%

Table 3. Test agreement of various *Porcine circovirus-2* serologic assays on serum samples collected from pigs in 6 pens each of 3 different sites over time*

	INGEZIM ELISA (1:100 dilution)		SERELISA (1:10 dilution)		ISU ELISA (1:100 dilution)	
	Positive	Negative	Positive	Negative	Positive	Negative
IFA (1:5 dilution)						
Positive	6	46	21	31	49	3
Negative	0	38	3	35	19	19
% agreement† (k value)	49 (0.099)		62 (0.296)		76 (0.469)	
	INGEZIM ELISA (1:100 dilution)		SERELISA (1:10 dilution)		IFA (1:5 dilution)	
	Positive	Negative	Positive	Negative	Positive	Negative
ISU ELISA (1:100 dilution)						
Positive	6	62	22	46	49	19
Negative	0	22	0	22	3	19
% agreement† (k value)	31 (0.059)		49 (0.189)		76 (0.469)	

*Each serum represents a pool of 5 pig sera per pen at each sampling time. ISU ELISA = Iowa State University-developed enzyme-linked immunosorbent assay; IFA = indirect fluorescent antibody test.
† % agreement = [agreed pos + neg/total tests (18 pens × 5 sampling points = 90)] × 100.

agreement in test results ($\kappa = 0.469$), suggesting a moderate level of agreement. The lowest agreements in test results were observed between IFA and INGEZIM ELISA (49% agreement, $\kappa = 0.099$) and between ISU ELISA and INGEZIM ELISA (31% agreement, $\kappa = 0.059$).

Detection of PCV2-specific antibody in oral fluid samples

Because a low level of antibody was expected in oral fluid samples, both a lower dilution (1:5) and the recommended dilution (i.e., 1:10 for SERELISA and 1:100 for ISU ELISA) of each oral fluid sample were tested by IFA, SERELISA, and ISU ELISA. INGEZIM ELISA was not used because of its low sensitivity in detecting anti-PCV2 antibody in serum. As overall ELISA results correlated better with IFA results at the lower dilution of oral fluid samples, which was also the case for the serum samples, all results described below for oral fluid samples were from the assays using the modified procedure (i.e., low dilution).

Similar to the results on the pooled serum samples, PCV2-specific IgG was detected in oral fluid samples with decreasing antibody titer until 8 weeks of age by all of the tests (Table 4, Fig 2B, D, F). However, the number of positive pens was less than that determined based on the pooled serum samples, and the level of antibody in oral fluid samples was generally lower than that in the serum samples (Fig 2). None of the oral fluid samples collected from pigs in all 3 sites at 12 weeks of age was positive for PCV2 IgG except for 1 pen in site 2, which was positive by IFA (Table 4). While pigs in most of the pens in all sites became seropositive for PCV2 at 16 weeks of age, only the oral fluid samples collected from all pens in site 1 and 1 pen in site 2 were positive for PCV2-specific IgG at the same age. Anti-PCV2 IgA was detected by IFA in oral fluid samples collected from all pens at 16 weeks of age that were also positive for anti-PCV2 IgG.

Comparison of assay performance on oral fluid samples

Using a lower dilution, a good agreement in test results was observed between IFA and SERELISA (93% agreement; $\kappa = 0.87$) and between IFA and ISU ELISA (88% agreement, $\kappa = 0.75$; Table 5). The recommended dilution (1:100) for ISU ELISA on serum samples seriously impaired the sensitivity of the test for detecting IgG antibody specific for ORF2 product of PCV2 in oral fluid samples. In contrast, results of SERELISA were not greatly influenced by the dilution factor. The

Table 4. Detection of *Porcine circovirus-2*-specific antibodies in oral fluid samples by various serologic assays over time after placement of animals in 1 of 3 different sites*

Age (weeks)	Site 1				Site 2				Site 3			
	IFA		SERELISA	ISU ELISA (IgG)	IFA		SERELISA	ISU ELISA (IgG)	IFA		SERELISA	ISU ELISA (IgG)
	IgG	IgA			IgG	IgA			IgG	IgA		
3	6/6	0/6	6/6	6/6	6/6	0/6	6/6	6/6	6/6	0/6	6/6	6/6
5	1/6	0/6	1/6	1/6	5/6	0/6	4/6	5/6	4/6	0/6	5/6	5/6
8	0/6	0/6	0/6	0/6	4/6	0/6	4/6	4/6	1/6	0/6	1/6	1/6
12	0/6	0/6	0/6	0/6	1/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
16	6/6	6/6	6/6	0/6	1/6	1/6	0/6	0/6	0/6	0/6	0/6	0/6

*Number of positive pens/total number of pens tested (n = 6). At each sampling time (age), oral fluid samples were collected from pigs in 6 different pens per site. Each oral fluid sample represents a pen. All samples were tested after diluted 1:5. IFA = indirect fluorescent antibody test; ISU ELISA = Iowa State University-developed enzyme-linked immunosorbent assay.

Table 5. Test agreement of various *Porcine circovirus-2* assays on oral fluid samples collected from pigs in 6 pens each of 3 different sites over time. Each oral fluid sample represents a pen at each sampling time*

	SERELISA (1:5)†		ISU ELISA (1:5)†		SERELISA (1:10)‡		ISU ELISA (1:100)‡	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
IFA (1:5)								
Positive	37	4	32	9	34	7	6	35
Negative	2	47	2	47	0	48	0	49
% agreement§ (k value)	93 (0.865)		88 (0.75)		92 (0.84)		61 (0.157)	

*ISU ELISA = Iowa State University-developed enzyme-linked immunosorbent assay; IFA = indirect fluorescent antibody test.
†Numbers in parentheses describe the dilution of samples for each assay, which is lower than what was recommended by the manufacturer or developer.
‡Numbers in parentheses describe the dilution of samples for each assay as recommended by the manufacturer or developer.
§ % agreement = [agreed pos + neg/total tests (18 pens × 5 sampling points = 90)] × 100.

recommended dilution (1:10) for SERELISA on serum samples slightly reduced the sensitivity of the test, as 92% agreement was observed between IFA and SERELISA.

Discussion

Oral fluid samples have been successfully used to detect antibodies specific for several pathogens in both human and veterinary medicine, particularly when sample collection and test procedure were optimized (7,12,28). Advantages of oral fluid sampling over serum sampling are ease of collection, noninvasiveness in sample collection, and representation of population. Despite these advantages, a few disadvantages have been identified with using oral fluid samples: a) a relatively low concentration of antibody compared to serum; b) the tendency of rapid antibody or agent degradation by bacterial growth and proteolytic enzyme breakdown; and c) lack of standardized antibody detection procedure (9,11). To circumvent these difficulties, modifications (e.g., pretreatment of samples, and different dilution of sample) need to be made in test procedures to optimize the analytic and diagnostic performance of the tests. In the current study, oral fluid samples were tested at a lower dilution (1:5) than recommended (i.e., 1:10 or 1:100 for SERELISA or ISU ELISA, respectively). With such a modification, IFA, SERELISA, and ISU ELISA showed a relatively good agreement regardless of whether they were performed on serum (49-76% agreement; $\kappa = 0.19$-0.47) or oral fluid samples (88-93%; $\kappa = 0.75$-0.87). Nevertheless, the assays demonstrated differences in their sensitivities, which could be attributed to a difference in the ability of each assay to detect immunoglobulins of different isotypes or subclasses (5) and/or antibodies against different epitopes of PCV2. A better agreement was observed among the assays when oral fluid samples were tested instead of serum. It might be because more defined isotypes or subclasses of antibodies would be transferred to oral fluids from blood circulation (17). In contrast, INGEZIM ELISA showed a poor agreement with the other assays used in the present study probably due to low sensitivity of the assay as compared to the IFA. Receiver operating characteristic (ROC) analyses suggested that test performance of INGEZIM ELISA could be improved if the cut-off (0.213) of the assay for IgG was lowered. If 0.032 was to be used as a cut-off, the sensitivity and specificity of the assay would be 96% and 92%, respectively, as compared to IFA results (IgG) on the serum samples (data not shown).

The serum PCV2 viremia and antibody profiles observed in the current study are similar to reports by other investigators even though sera were tested after being pooled. Previous field studies (19) demonstrated that maternal antibody specific for PCV2 gradually decayed by 11 weeks of age, and seroconversion to the virus occurred again between 12 and 15 weeks when PCV2-positive farms were monitored from 3 up to 28 weeks of age. In those herds, PCV2 viremia was detected between 11 and 15 weeks of age (30). In agreement with previous reports, the current study also demonstrated that PCV2-specific maternal antibody continued to decay and was undetectable between 8 and 12 weeks of age in both serum and oral fluid samples. Seroconversion to the virus was observed at 16 weeks of age (Table 2, Fig 2) regardless of the type of sample tested (i.e., serum or oral fluid samples), suggesting that the study sites were experiencing recent infection by PCV2. The recent PCV2 infection was substantiated by detecting a high level of PCV2 viremia in pigs at 12 weeks of age and PCV2-specific IgA and/or IgM antibodies besides IgG in oral fluid and serum samples at 16 weeks of age (Figs. 1, 3B). Levels of PCV2 DNA in oral fluid samples were similar to those in pooled serum over time with a correlation coefficiency rate (r^2) of 0.61 (Fig 1). Furthermore, the antibody kinetic profile over time, which was determined using oral fluid samples, was also similar to that determined using sera (Fig 2). Therefore, the general profile reflecting the herd status of PCV2 infection/circulation and humoral immunity were compatible regardless of whether serum or oral fluid sample was used for the evaluation.

Observations from the present study suggest that oral fluids can be an alternative to serum for detecting PCV2 DNA and/or PCV2-specific antibody at a population level. Since PCV2 has been reported to replicate in the tonsil (3), oral fluids might be a better material to detect PCV2 at the early stage of infection. The present study demonstrated earlier detection of PCV2 circulation in all of the study sites when oral fluid samples were tested in comparison to serum samples (Table 1), suggesting that oral fluid samples could be good for prognostic herd profiling. Levels of PCV2 in oral fluid samples collected at between 3 and 12 weeks of age were lower than those in samples collected at 16 weeks of age (Fig 1). Such low levels of PCV2 in oral fluid samples could reflect a low prevalence of infected pigs in each of the study sites or environmental contamination as the virus is extremely stable (25). This observation may emphasize the importance of performing a quantitative assay for PCV2 using oral fluid samples to determine whether or not active PCV2 infection is established in pigs. With selection of proper tests and appropriate adjustments of test procedures, oral fluid sampling may be a cost-effective way to monitor virus activity or immune status in a swine population.

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구강액을 이용한 양돈장의 *Porcine circovirus-2* 감염에 대한 모니터링

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요 약 : *Porcine circovirus-2* (PCV2) 는 돼지에서 여러 형태의 질병과 증후군의 발생과 관련이 되어있어 현재는 PCV-associated diseases (PCVAD)로 총괄적으로 분류된다. PCVAD에 의한 높은 경제적 손실 때문에 많은 양돈장들이 PCV2의 감염을 확인하기 위하여 혈청을 검사하고 있다. 하지만, 기존의 혈액채취법은 비용이 높고 많은 인력이 소요되므로 큰 규모의 병원체 검사에는 어려움이 있었다. 이에 본 연구에서는 혈액채취법을 이용한 돈군의 PCV2 검사법에 대한 대체방법으로 돈방 단위의 구강액채취법의 유용성을 실제 농장에서 평가하였다. 세 곳의 다른 양돈 농장들에서 각각 6개의 25두 규모의 돈방들을 선정하여 생후 3, 5, 8, 12, 16주에 돈방 마다 하나의 구강액과 5개의 혈청을 채취하였다. 모든 시료들은 real-time PCR을 이용하여 PCV2 DNA를 검사하였고 IgG 또는 IgA 간접형광항체 검사법 및 세 가지의 ELISA 검사법 (blocking ELISA, indirect ELISA, and IgG/IgM sandwich ELISA)을 이용하여 PCV2에 대한 항체를 검사하였다. 구강액에서 PCV2 DNA는 8주까지는 간헐적으로 검출이 되다가 16주에는 모든 돈방에서 검출이 되었으며, 모체유래 PCV2 특이 IgG는 3주부터 검출이 되었고 모든 농장에서 5-8주까지 지속이 되었다. 16주에는 한 농장 (Site 1)의 모든 돈방에서 감염에 의한 IgG와 IgA가 검출되었다. 혈청에서의 PCV2 DNA와 PCV2 항체의 검출은 구강액에서의 검출과 높은 상관관계를 보였다. 따라서 구강액은 돈군의 PCV2 감염을 모니터링 하기 위해 혈청대신 사용할 수 있는 저비용, 고효율의 시료가 될 수 있을 것으로 사료된다.

주요어 : 구강액, *Porcine circovirus-2*, 항체, enzyme-linked immunosorbent assay, 간접형광항체법