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RESEARCH ARTICLE

Comparative Study of Two Point-of-Care Enzyme-Linked Immunosorbent Assays for the Detection of Antibodies against Canine Parvovirus and Canine Distemper Virus

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ABSTRACT

This study was conducted to compare two commercially available point-of-care enzyme-linked immunosorbent assay kits (TiterCHEK® and ImmunoComb®) for the detection of antibodies against CPV and CDV. Dogs were clinically healthy, and included 22 (21%) beagles and 83 (79%) Korean native bobtailed dogs admitted to an animal shelter. Of the 105 dogs, 89 (84.76%) were found positive for CPV protective antibody titers (PATs) by HI assay and 3 (2.85%) were identified as positive for CDV PATs by SN assay. The CPV PAT was significantly higher (Pearson correlation coefficient=0.297, P=0.002) in adult dogs (≥1 year) than that in young dogs (<1 year). For an accurate identification of CPV and CDV PATs, although not statistically significant, the specificity (100% for both CPV and CDV) and positive predictive values (PPV, 100% for both CPV and CDV) of the TiterCHEK were higher than those of the ImmunoComb (specificities for CPV and CDV, 81.3% and 97.1%; PPV for CPV and CDV, 81.3% and 97.1%, respectively). The TiterCHEK had fewer false-positive results than the ImmunoComb. To the best of our knowledge, this is the first study that shows compare sensitivity and specificity between the TiterCHEK® and ImmunoComb® kits, using CPV HI and CDV SN as reference tests.

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INTRODUCTION

The canine parvovirus (CPV) and canine distemper virus (CDV) are most common causes of infectious disease in dogs worldwide (Twark and Dodds, 2000; Sykes and Rankin, 2013; Jensen *et al.*, 2015). Two viruses can be a major cause of mortality and morbidity in unvaccinated dogs in animal shelters, pet shops and puppy mills, and in dogs that have poor antibody formation after vaccination (Beineke *et al.*, 2009; Goddard and Leisewitz, 2010; Steneroden, 2011). Currently, CPV infection and canine distemper have no effective medicine to treat (Lamm and Rezabek, 2008; Martella *et al.*, 2008). If infected, clinical signs of dogs with CDV can occur as acute or subacute as a generalized infection, gastro-intestinal and respiratory sign, the signs within the central nervous system, or a combination of these (Cha *et al.*, 2012). Clinical signs of dogs with CPV may include lethargy, anorexia, vomiting, diarrhea, nausea, leucopenia, and myocarditis (Miranda *et al.*, 2015; Yang *et al.*, 2015). Therefore, the sole method to preventing CPV and CDV outbreaks is vaccinations (Hurley, 2009; Day *et al.*, 2016). Antibodies can get maternal antibody, naturally or through vaccination and infections (Sykes and Rankin, 2013). Antibody titers are measuring the amount of antibody in the bloodstream that is produced in response to infection or vaccination against CPV and CDV. Through antibody titer measurements against the CPV and CDV, it can play a role as a guide for vaccination (Tizard and Ni, 1998), the effectiveness of vaccination (Eghafona *et al.*, 2007; Jensen *et al.*, 2015) and infection in the past (Mouzin *et al.*, 2004; Schultz, 2006; Lechner *et al.*, 2010; Litster *et al.*, 2012). Serum CPV and CDV titers can be measured by hemagglutination inhibition (HI) and serum neutralization (SN) tests (Desario *et al.*, 2005; Newbury *et al.*, 2009; Lecher *et al.*, 2010), as well as enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody (IFA) test (Twark and Dodds, 2000; Waner *et al.*, 2003; Sykes and Rankin, 2013; Jensen *et*

al., 2015). HI is the reference standard for determining serum CPV titers, and SN is the reference standard for determining serum CDV titers (Twark and Dodds, 2000; Jensen *et al.,* 2015).

At present, 2 in-clinic ELISA kits are approved for sale in many countries including the South Korea and United States, the TiterCHEK CDV/CPV kit (TiterCHEK®, Synbiotics Corp., San Diego, CA, USA) and the ImmunoComb Canine VacciCheck IgG Antibody Test Kit (ImmunoComb®, Biogal Galed Laboratories, Kibbutz Galed, Israel). In clinics, the two point-of-care ELISA kits (TiterCHEK (Lechner *et al.,* 2010; Gray *et al.,* 2012; Litster *et al.,* 2012; Litster *et al.,* 2012) and ImmunoComb (Waner *et al.,* 2003; Waner *et al.,* 2006; Eghafona *et al.,* 2007; Mazar *et al.,* 2010; Acosta-Jamett *et al.,* 2015)) are used to rapidly and easily detect serum antibodies against CPV and CDV in dogs. However, up to the present time, differences between the TiterCHEK and ImmunoComb kits have not been evaluated. The aim of this study was to compare the sensitivity and specificity between the TiterCHEK and ImmunoComb kits through detection of CPV and CDV antibody titers using sera of dogs in animal shelter.

MATERIALS AND METHODS

Animals and sample collection: This study included 105 healthy dogs that had been admitted to an animal shelter. A determination of clinical health was based on physical examination by a veterinarian. The experimental design of this study was approved by the University of Konkuk Institutional Animal Care and Use Committee. Blood samples (3 mL) were collected via jugular or cephalic venipuncture from dogs (estimated age based on dental examination of ≥3 months' age to minimize the impact of maternally-derived antibodies) (Sykes JE and Rankin SC, 2013). Blood samples were centrifuged to obtain serum. All samples were assayed by two ELISA kits and reference standard of HI and SN titers against CPV and CDV.

'Gold standard' measurement of CPV HI and CDV SN titers: CDV SN and CPV HI titers were determined at a diagnostic laboratory using reference tests (Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University, Ithaca, NY). SN titers against CDV were expressed as the reciprocal of the highest dilution of serum that neutralized infectivity of the virus (e.g., endpoint dilution of 1:32=antibody titer of 32). For CDV, the diagnostic laboratory reported the PAT to be in the range of 32–1024. The HI titer against CPV was the reciprocal of the highest dilution of serum that prevented the agglutination of red blood cells by parvovirus (e.g., endpoint dilution of 1:80=antibody titer of 80). The diagnostic laboratory reported the PAT to be in the range of 80-2560 for CPV by HI assay.

The TiterCHEK® CDV/CPV point-of-care ELISA test kit: The TiterCHEK CDV/CPV kit (TiterCHEK®, Synbiotics Corp., San Diego, CA, USA), an ELISA-based assay, was used to determine the levels of antibody against CDV and CPV in canine serum sample, following the manufacturer's instructions. Development of a blue color in the sample well of equal or greater intensity than the color of the positive control indicated a positive sample, corresponding to an anti-CPV antibody titer ≥80 (as determined by HI assay) or an anti-CDV antibody titer ≥16 (as determined by SN assay). Development of a blue color with less intensity than the color of the positive control indicated that a sample was negative (CPV HI titer <1:80 or CDV SN titer <1:16). Results of the TiterCHEK test were available within approximately 20 minutes.

The ImmunoComb® Canine VacciCheck IgG antibody point-of-care ELISA test kit: Another ELISA kit, the ImmunoComb Canine VacciCheck IgG Antibody Test Kit (ImmunoComb®, Biogal Galed Laboratories, Kibbutz Galed, Israel) was used to detect CPV and CDV antibody titers in dog serum, following the manufacturer's instructions (Mazar *et al.,* 2010). The concentration of CPV and CDV antibody titers of each sample was measured using a color-coded scale provided in the kit, and the results were expressed in "S units" on a scale from 0 to 6. An S value ≥3 was standardized by the manufacturer as equivalent to an HI antibody titer ≥80 and an SN antibody titer ≥1:32 for CPV and CDV, respectively. An S value of ≥3 was regarded as a PAT. Samples with S values of 3-6 were regarded seropositive. Results for the ImmunoComb test were obtained within 23 minutes.

Statistical analysis: The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy were calculated. McNemar's test was used to identify significant differences between the results of the ELISA kits and HI assays. The statistical analysis was performed using SPSS for Windows version 20.0 (IBM, Chicago, IL, USA). For all analyses, values of $P \leq 0.05$ were considered significant.

RESULTS

The dogs included 41 (39%) intact males and 64 (61%) intact females, ranging in age from 3 months to 4.3 years (mean±SD, 1.35±1.86 years), with 49.5% (52/105) <5 months old, 21% (22/105) 6-12 months old, and 29.5% (31/105) >12 months old. Overall profiles of comparative study among 3 different tests against CPV and CDV in 105 dogs were summarized in Table 1. The CPV PAT positivity rate in adult dogs (≥1 year old, 2.44±1.06 years) was higher (P=0.005) than that in young dogs (<1 year old, 0.58±0.26 year) (Table 2). Of the 105 dogs tested, 89 (84.8%) were found to have PATs against CPV, as determined by HI assay, and 3 (2.86%) were identified as having PATs against CDV, based on SN assay (Table 3). Three dogs had both CDV and CPV PATs. The prevalence of CPV PATs was significantly higher (Pearson correlation coefficient = 0.297, P=0.002) in adult dogs (≥1 year) than that in young dogs (<1 year). All dogs older than 1 year (31/31) had CPV PAT and 78.38% (58/74) of dogs under 1 year had CPV PAT (Fig. 1A). All three dogs with CDV PAT were all under 1 year old (Fig. 1B).

Compared to the reference assays, the TiterCHEK had higher specificity for CPV (100%) and CDV (100%)

than did the ImmunoComb (81.3% and 97.1%, respectively); the sensitivity for CPV was similar (TiterCHEK, 88.8%; ImmunoComb, 87.6%) and the sensitivity for CDV was same (both 100%); PPV for CPV (100%) and CDV (100%) with the TiterCHEK were higher than with the ImmunoComb (96.2% and 50%, respectively); overall accuracy for CPV (90.5%) and CDV (100%) with the TiterCHEK were slightly higher than with the ImmunoComb (86.7% and 97.1%, respectively) (Table 4). However, the sensitivity (P=0.99 for CPV), specificity (P=0.23 for CPV), PPV (P=0.24 for CPV), NPV (P=0.81 for CPV), and overall accuracy (P=0.51 for CPV) of TiterCHEK were not significantly different from those of the ImmunoComb in identifying dogs with antibodies against CPV. The statistical analyses for CDV were not performed because the number of CDV antibody positive serum was too small to draw any statistical analysis. Based on the above results, there is no significant difference between the TiterCHEK and ImmunoComb.

Table 1: Overall profiles of comparative study among 3 different tests against canine distemper virus (CDV) and canine parvovirus (CPV) in 105 dogs

Sample	CDV Ab result			CPV Ab result			Sample	CDV Ab result			CPV Ab result		
No.	A	B	C	A	B	D	No.	A	B	C	A	B	C
1	0	0	<4	5	1	640	54	1	0	4	5	1	160
2	0	0	<4	5	1	160	55	0	0	<4	1	0	10
3	0	0	<4	4	1	640	56	1	0	<4	3	0	20
4	0	0	<4	5	1	1280	57	0	0	<4	5	1	640
5	0	0	<4	3	1	640	58	2	0	<4	4	1	320
6	0	0	<4	3	1	640	59	2	0	<4	5	0	1280
7	0	0	<4	5	1	2560	60	0	0	<4	2	0	40
8	0	0	<4	4	1	1280	61	1	0	<4	5	1	640
9	0	0	<4	3	1	640	62	0	0	<4	5	1	2560
10	0	0	<4	4	1	1280	63	2	0	<4	5	1	2560
11	0	0	<4	5	1	1280	64	2	0	<4	5	1	2560
12	0	0	<4	5	1	640	65	2	0	<4	5	1	640
13	0	0	<4	0	0	20	66	1	0	<4	5	1	2560
14	0	0	<4	0	0	80	67	2	0	<4	5	1	1280
15	0	0	<4	1	1	320	68	2	0	<4	5	1	1280
16	0	0	<4	0	0	20	69	0	0	<4	5	1	1280
17	0	0	<4	3	1	640	70	2	0	<8	5	1	640
18	0	0	<4	4	1	1280	71	2	0	<4	5	1	320
19	0	0	<4	4	1	1280	72	0	0	<4	5	1	160
20	0	0	<4	5	1	5120	73	0	0	4	4	1	160
21	0	0	<4	0	0	20	74	1	0	<4	4	1	160
22	0	0	<4	0	0	40	75	0	0	12	5	1	160
23	0	0	<4	0	0	80	76	0	0	<4	4	1	80
24	0	0	<4	0	0	40	77	0	0	4	5	1	80
25	0	0	<4	3	1	80	78	0	0	<4	5	0	40
26	0	0	<4	3	1	160	79	0	0	<4	4	0	40
27	0	0	<4	2	0	80	80	2	0	<4	5	1	1280
28	0	0	<4	3	1	80	81	0	0	<4	6	1	640
29	0	0	<4	2	1	80	82	0	0	<4	6	1	1280
30	0	0	<4	2	1	160	83	1	0	<4	6	1	320
31	0	0	<4	2	1	80	84	0	0	<4	0	0	80
32	1	0	<4	5	1	640	85	0	0	<4	1	0	80
33	0	0	<4	4	1	2560	86	0	0	<4	0	0	10
34	1	0	<4	4	1	1280	87	0	0	<4	0	0	40
35	3	0	<4	5	1	1280	88	0	0	8	0	0	40
36	0	0	<4	4	1	640	89	0	0	<4	0	0	80
37	2	0	<4	4	1	320	90	0	0	4	3	0	640
38	0	0	<4	4	1	1280	91	0	0	<4	0	0	80
39	0	0	<4	4	1	1280	92	0	0	4	0	0	20
40	0	0	<4	4	1	640	93	0	0	<4	3	0	80
41	0	0	<4	5	1	2560	94	2	0	16	5	1	5120
42	0	0	<4	4	1	160	95	2	0	<8	5	1	2560
43	0	0	<4	4	1	2560	96	3	1	48	5	1	2560
44	0	0	<4	3	1	160	97	2	0	8	5	1	5120
45	0	0	<4	5	1	1280	98	3	0	<8	5	1	1280
46	0	0	<4	4	1	320	99	3	0	12	5	1	2560
47	0	0	<4	4	1	320	100	5	1	768	5	1	640
48	1	0	<4	4	1	1280	101	0	0	<4	5	1	2560
49	0	0	<4	5	1	1280	102	0	0	<4	5	1	5120
50	0	0	<4	5	1	320	103	0	0	<4	5	1	5120
51	0	0	<4	5	1	1280	104	5	1	1024	4	1	2560
52	0	0	<4	1	0	20	105	0	0	<4	4	1	1280
53	0	0	<4	1	0	20							

A: ImmunoComb®, Biogal Lab, negative: <3, positive: 3-6; Results are expressed in "S units" on a scale based on a color change from S0-S6 and are interpreted next to a control always considered a S3. An equal or darker color tone (S3-S6) than the reference spot is considered a positive result (HI titer ≥1:80 for CPV, SN titer ≥1:32 for CDV). A faint color tone (S0-S2) is considered a negative result (HI titer <1:80 for CPV, SN titer <1:32 for CDV). B: TiterCHEK®, Zoetis Inc., negative: 0 (SN titer <1:16 for CDV, HI titer <1:80 for CPV), positive: 1 (SN titer ≥1:32 for CDV, and HI titer ≥1:80 for CPV). C: Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University, SN titer for CDV, negative: <32, positive: 32-1024. D: Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University, HI titer for CPV, negative: <80, positive: 80-2560.

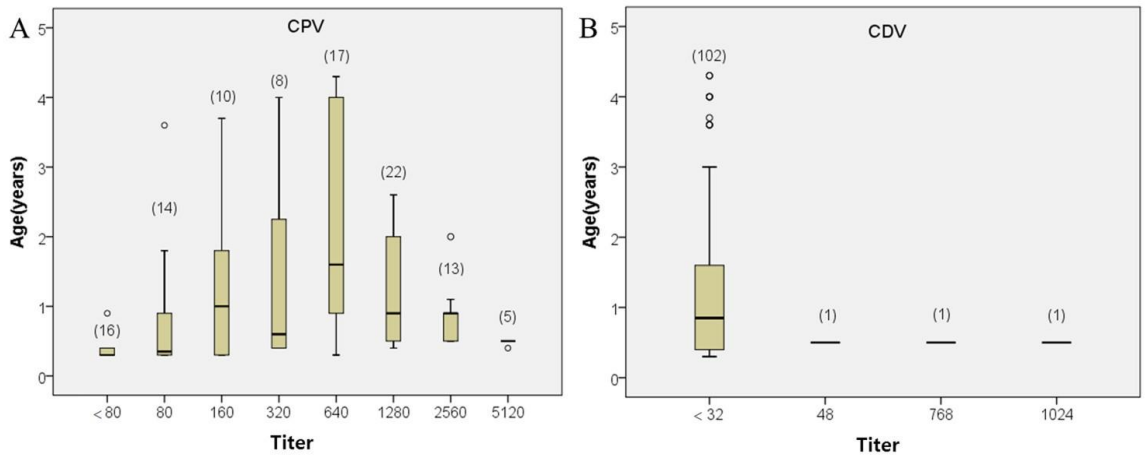

Fig. 1: Box plot showing the relationship between the age of dogs and serum antibody titers against CPV (A) and CDV (B) determined by hemagglutination inhibition (HI) and serum neutralization (SN). The number in parentheses is the number of dogs testing positive for antibody. Outliers are indicated by circles. HI titer for CPV, negative: <80, positive: 80–5120. SN titer for CDV, negative: <32, positive: 32-1024.

Table 2: Association between various factors and the presence of protective antibody titers (PATs) against canine distemper virus (CDV) and canine parvovirus (CPV) determined by serum neutralization and hemagglutination inhibition assay in 105 dogs

Variables		No. (%) of dogs tested	No. (%) of dogs with PAT to CPV (n=89)	P	No. (%) of dogs with PAT to CDV (n=3)†
Age	Young	74 (70.48)	58 (78.38)	0.005*	3 (4.05)
	Adult	31 (29.52)	31 (100)		0
Gender	Male	47 (44.76)	43 (91.49)	0.084	2 (4.26)
	Female	58 (55.24)	46 (79.31)		1 (1.72)
Breeds	KND	83 (79.05)	71 (85.54)	0.667	0
	Beagle	22 (20.95)	18 (81.81)		3 (13.63)

Young, <1 year; Adult, ≥1 years; PAT, protective antibody titer; KND, Korean native bobtailed dog; No., number. †This number is too low for any meaningful statistical analyses to be done. Thus, the statistical analysis was not performed. * Values of P≤0.05 were considered significant.

Table 3: Comparison of results of the hemagglutination inhibition assay as a reference test for canine parvovirus antibody and the serum neutralizing assay as a reference test for canine distemper virus with results of the TiterCHEK and ImmunoComb test kits using sera from 105 dogs

Test performed		HI for CPV antibody (n=105)			SN for CDV antibody (n=105)	
		Positive	Negative		Positive	Negative
TiterCHEK	Positive	79	0	Positive	3	0
	Negative	10	16	Negative	0	102
ImmunoComb	Positive	78	3	Positive	3	3
	Negative	11	13	Negative	0	99

TiterCHEK: TiterCHEK® CDV/CPV kit, Synbiotics Corp, San Diego, CA, USA; ImmunoComb: ImmunoComb® Canine VacciCheck IgG Antibody Test Kit, Biogal Galed Laboratories, Kibbutz Galed, Israel; HI, hemagglutination inhibition of CPV, negative: < 80, positive: 80–2560; SN, serum neutralizing titer against CDV, negative: <32, positive: 32–1024.

Table 4: Calculated sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy of two enzyme-linked immunosorbent assay in detecting antibody against canine parvovirus (CPV) and canine distemper virus (CDV), using hemagglutination inhibition (HI) and serum neutralization (SN) as reference standards

Values	HI for CPV antibody		SN for CDV antibody	
	TiterCHEK	ImmunoComb	TiterCHEK	ImmunoComb
Sensitivity (%, 95% CI)	88.8 (80.3-94.5)	87.6 (78.8-93.7)	100 (29.2-100)	100 (29.2-100)
Specificity (%, 95% CI)	100 (79.4-100)	81.3 (54.3-96.0)	100 (96.5-100)	97.1 (91.7-99.4)
PPV (%, 95% CI)	100 (95.4-100)	96.2 (89.5-99.2)	100 (29.2-100)	50.0 (11.8-88.2)
NPV (%, 95% CI)	61.5 (40.6-79.8)	54.2 (32.9-74.5)	100 (96.5-100)	100 (96.3-100)
Overall accuracy (%)	90.5	86.7	100	97.1

TiterCHEK: TiterCHEK® CDV/CPV kit, Synbiotics Corp, San Diego, CA, USA; ImmunoComb: ImmunoComb® Canine VacciCheck IgG Antibody Test Kit, Biogal Galed Laboratories, Kibbutz Galed, Israel. Sensitivity, true positive results/(true positive results + false negative results); specificity, true negative results/(true negative results + false positive results); PPV, positive predictive values, true positive results/(true positive results + false positive results); NPV, negative predictive values, true negative results/(true negative results + false negative results); overall accuracy, (true positive results + true negative results)/all results; Ab, antibody; HI, hemagglutination inhibition; SN, serum neutralization; 95% CI, 95% confidence interval. *Values of P≤0.05 were considered significant.

DISCUSSION

CPV PAT by reference standard were significantly higher (Fisher's Exact Test, P<0.0001) than CDV PAT. These results showed similar results in previous studies (Lechner *et al.*, 2010; Gray, 2012; Litster *et al.*, 2012). A primary reason for this difference in antibodies against the two viruses may be the durability of CPV, which can survive for prolonged periods (over 1 year) in the environment, providing more opportunities for natural exposure to dog hosts (McCaw and Hoskins, 2006; Sykes and Rankin, 2013). In contrast, CDV shows poor survival in the environment (surviving less than 1 day at room temperature) and is readily inactivated by heat, drying, and exposure to disinfectants; thus, there may be less opportunity for dogs to acquire immunity by exposure to

contaminated environments (Greene and Appel, 2006; Sykes and Rankin, 2013). The detection rate of CDV PAT was very low. Considering about these result, an inadequate antibody response to CPV and CDV in a dog could mean any of the following (Sykes and Rankin, 2013): 1) exposure to the pathogen of interest did not occur, 2) serum was sampled too early in course of infection/illness, 3) severe immunosuppression in that dog, 4) poor analytical sensitivity of the assay, 5) or lack of vaccination.

The CPV PAT in adult dogs (≥1 year old) was significantly higher (P=0.005) than that in young dogs (<1 year old). In previous study, the percent of dogs showing PATs against CPV and CDV was higher in adult dogs than in young dogs (Lechner *et al.*, 2010; Taguchi *et al.*, 2011; Acosta-Jamett *et al.*, 2015). Older dogs may have had more time for exposure to vaccines or natural infection. In this study, most dogs without CPV PAT were 5 months of age. Puppies are a loss of PAT due to decrease of maternal antibody concentration and susceptible to virus infection (Sykes and Rankin, 2013). The WSAVA Vaccination Guidelines Group recommends vaccination of core vaccine to puppies over 16 weeks of age (Day *et al.*, 2016).

The sex of dogs (male or female) and breed (Beagle or Korean native bobtail dog) were not associated with the presence of CPV PATs. However, CDV PATs were more frequently detected (P=0.001) in beagles than in Korean native bobtail dogs. However, it seems hard to give meaning because the differences between the results of the two breeds was minor (Table 2).

The TiterCHEK showed higher sensitivity, specificity, PPV, NPV and overall accuracy in the detection of PAT against CPV than did the ImmunoComb (Table 4). In addition, TiterCHEK showed a higher specificity and PPV in identifying dogs with CDV PAT than did ImmunoComb, resulting in fewer false positive results with TiterCHEK (Table 4). The sensitivities and NPVs for detecting CDV antibodies by TiterCHEK and ImmunoComb kits, however, were same. Compare to previous assessments of TiterCHEK (Gray *et al.*, 2012; Litster *et al.*, 2012), sensitivity of this study has shown lower for CPV (88.8% vs 92.3-98%) and higher (100% vs 75.7-88%) for CDV than those of previous study; specificity have shown both lower for CPV (100% vs 93.5-98%) and CDV (100% vs 91.8-95%). Compare to previous assessments of ImmunoComb by the manufacturer provided study (Mazar *et al.*, 2010), sensitivity (97% vs 87.6%) and specificity (100% vs 81.3%) for CPV have shown both higher than those of our study; sensitivity (95% vs 100%) for CDV have shown mild lower and specificity (100% vs 97.1%) for CDV have shown mild higher. The point-of-care ELISA results for CPV and CDV PATs were a slight difference each other. However, the CDV analyses were meaningless given the small positive numbers. In the CPV analyses, with a relative sensitivity of less than 90% and an overall accuracy of just 90.5%, it is quite risky for clinicians to decide whether to vaccinate or not based on these tests. The sample size of this study is rather small. In the previous comparative study (Gray *et al.*, 2012), the samples were evaluated using 431 sera. Therefore, samples of large sizes would be helpful for more accurate evaluation of both ELISA kits. In general, point-of-care ELISA kit have inherent limitations such as false positive or false negative results in serological assays (Litster *et al.*, 2012). A false positive result may occur because of substantial amount of antibodies in the specimen binding nonspecifically to the antigen spot in the kit. False negative results may occur due to low ELISA sensitivity, especially when PAT does not induce sufficient protection from challenge and vaccination. Except for some presented samples, HI antibody concentrations of most samples with the false negative result had low margin value among positive determination range.

Each point-of-care ELISA kits have characteristics, including the multistep procedures, testing time, sample volume required for test, and cost of kit (Waner *et al.*, 2003; Eghafona *et al.*, 2007; Gray *et al.*, 2012; Litster *et al.*, 2012). At the time this study was accompanied, the ImmunoComb kit cost approximately $5.3/sample, except labor costs. And, the ImmunoComb kit requires a sample volume of 5-10 μL and 25 minutes to complete the procedures. However, interpretation of test result is slightly difficult: evaluated by comparing color results that developed in reference spot and in sample tested spot (Waner *et al.*, 2003). In contrast, the TiterCHEK kit requires only 1 μL of serum or plasma samples and within approximately 20 minutes, and it is relatively easy to distinguish between the color intensities of positive or negative control wells. However, the TiterCHEK kit cost approximately $15.3/sample is more expensive and is more difficult to perform the procedures, which required more complicated washing steps than the ImmunoComb assay, and required a skilled technician. Thus, veterinary diagnostic laboratories, animal shelters, or referral veterinary hospitals dealing with a large volume of serum samples seem to prefer using TiterCHEK, because less time and smaller sample volume are needed than required by ImmunoComb. However, if an institution routinely tests 12 sera, ImmunoComb seems to be preferred. Use of point-of-care ELISA kits may be measured with less cost, small sample volume, and brief time (within 1 hour) than inspection with the diagnostic laboratory (Gray *et al.*, 2012). It may be help determine whether PAT formation and antibody titer for revaccination against CPV and CDV in dogs (Eghafona *et al.*, 2007; Gray *et al.*, 2012; Litster *et al.*, 2012; Litster *et al.*, 2012).

Conclusions: By comparing measurements in serum antibody titers, the TiterCHEK and ImmunoComb were not significantly different with SN titer and HI titer analysis screening antibodies against CDV and CPV. Therefore, both ELISA kits could be useful test to detect anti-CDV and anti-CPV antibodies under field conditions and shelters, where infectious diseases outbreak. Although each examination has differences due to several factors including sample volume for test, test cost, test time, and test procedure of ELISA kits, this result could be helpful to choose the diagnostic assay in detecting antibody titer against CDV and CPV in veterinary field.

Authors contribution: SGK and HMP designed and supervised the study. MHK helped collect blood samples. SGK and MHK executed the experiment and analyzed the test results. The manuscript was prepared by KSG and

MHK under the supervision of HMP. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final manuscript.

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