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Accuracy of a point-of-care ELISA test kit for predicting the presence of protective canine parvovirus and canine distemper virus antibody concentrations in dogs

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ABSTRACT

Canine parvovirus (CPV) and canine distemper virus (CDV) are highly infectious and often fatal diseases with worldwide distributions, and are important population management considerations in animal shelters. A point-of-care ELISA test kit is available to detect serum antibodies to CPV and CDV, and presumptively to predict protective status. The aim of this study was to determine the diagnostic accuracy of the test compared to CPV hemagglutination inhibition titers and CDV serum neutralization titers determined by a reference laboratory, using sera collected from dogs housed at animal shelters. The ELISA test was used under both field and laboratory conditions and duplicate specimens were processed using an extra wash step.

The test kit yielded accurate results (CPV: sensitivity 92.3%, specificity 93.5%; CDV: sensitivity 75.7%, specificity 91.8%) under field conditions. CDV sensitivity was improved by performing the test under laboratory conditions and using an optical density (OD) meter (laboratory performed 94.0%; OD 88.1%). Point-of-care ELISA testing for serum CPV and CDV antibody titers was demonstrated to be a useful tool for determining antibody status when making decisions regarding the need for CPV and/or CDV vaccination and also in animal shelters for population management.

Introduction

Canine parvovirus (CPV) and canine distemper virus (CDV) are highly infectious and often fatal canine infections with worldwide distributions (Beineke et al., 2009; Goddard and Leisewitz, 2010). Despite widespread vaccination, CPV and CDV remain major causes of morbidity and mortality, particularly in unvaccinated dogs housed in pet shops, puppy mills and animal shelters (Beineke et al., 2009; Goddard and Leisewitz, 2010; Steneroden et al., 2011). Although immunological resistance to CPV and CDV is multifactorial, moderately to markedly increased serum antibody responses distinguish resistant from susceptible animals (Krakowka et al., 1975; Noon et al., 1980; Carmichael et al., 1983; Winters et al., 1983; Rima et al., 1991). Therefore, measurement of serum antibody titers may be a useful tool for determining the need for vaccination (Tizard and Ni, 1998) and for making population management decisions in shelters (Lechner et al., 2010).

Predicting protective status using antibody titers may be challenging. Virulence of virus strain, size of challenge dose, and adequacy of T-helper cell-mediated immunity, cytotoxicity, and persistence of memory cells (which may prevent infection despite decline in serum antibody concentrations) are unpredictable variables when determining whether a dog is protected from disease. Additionally, recommendations for test interpretation are usually based on information from laboratory challenge studies, where the infective doses used may far exceed what is usual under field conditions. However, in a shelter or disease outbreak (where errors may place animals at unnecessary risk of disease) conservative interpretation of test results is needed to minimize this risk.

Serum CPV titers can be measured by ELISA, indirect fluorescent antibody assays (IFAs), or by hemagglutination inhibition (HI) or virus neutralization (VN) tests. CPV challenge studies have demonstrated an adequate antibody response to vaccination, and associated protection with an HI serum antibody titer $\geqslant$1:80 (Carmichael et al., 1983; Twark and Dodds, 2000). CDV titers are measured using ELISA, IFA and serum neutralization (SN) tests (Tizard and Ni, 1998). Early CDV challenge studies using SN determined that titers of 1:30–1:100 were shown to be protective (Gillespie, 1966; Appel, 1969), while a later study reported that titers of $\geqslant$1:32 (equivalent to an IFA result of $\geqslant$1:5) indicated a sufficient antibody response to vaccination (Twark and Dodds, 2000).

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The Synbiotics TiterCHEK CDV/CPV test[1] is a point-of-care ELISA test kit marketed for rapid determination of protective serum antibody concentrations in dogs against CPV and CDV (Carmichael, 2005). Results are interpreted as 'positive' or 'negative' for each virus, with the package insert claiming that a positive result for CPV indicates an antibody titer equivalent to a CPV HI titer of $\geqslant$1:80 and a positive for CDV indicates an antibody titer equivalent to a CDV SN titer of $\geqslant$1:16. The aim of the present study was to determine the sensitivity and specificity of the test kit, when compared to CPV HI titers and CDV SN titers measured by a reference laboratory, using sera collected from dogs housed at animal shelters.

Materials and methods

Serum samples

The study protocol was approved by the Purdue University Animal Care and Use Committee (PACUC No. 10-037). Sera were successively collected from dogs at the time of surrender or delivery by municipal authorities to two metropolitan animal shelters – PAWS Chicago, a large adoption-guarantee shelter, and the Humane Society of Indianapolis, a limited admission shelter. Enrollment criteria were (1) an estimated age based on dentition of $\geqslant$4 months age in order to minimize the confounding influence of maternally-derived antibodies (2011 American Animal Hospital Association Canine Vaccination Guidelines), and (2) clinically healthy animals based on physical examination by the attending shelter veterinarian. Dogs were excluded if estimated to be <4 months of age or if they had clinical signs of systemic illness at the time of shelter intake. A target enrollment of 50 dogs per shelter was used so that sample size would permit statistical analysis of results, with several extra convenience samples collected on the final study day.

Blood was collected at the time of shelter intake and the test was performed on site. After initial blood collection, each dog was vaccinated using a modified live C5 vaccine (PAWS Chicago, Pfizer Duramune Max 5; Humane Society of Indianapolis, Pfizer Vanguard Plus 5). All dogs that were suspected not to have protective titers against both CPV and CDV by the TiterCHEK CDV/CPV test kit performed on Day 1 were retested between Days 6 and 8. Dogs that may not have had protective titers against both CPV and CDV at the Days 6–8 recheck were retested between Days 13–15. Duplicate serum samples were collected and stored at −80 °C at each time point for (1) determination of CPV HI titers and CDV SN titers by a reference laboratory (Cornell University Animal Health Diagnostic Center), and (2) submission to the Synbiotics Corporation for TiterCheck testing under laboratory conditions by one laboratory technician and by microplate reader (ELx800 Universal Microplate Reader, Bio-Tek Instruments, Inc.).

Point-of-care ELISA test

The ELISA test was used according to the manufacturer's instructions, reporting results as either positive or negative. In brief, each assay includes separate CPV and CDV rows, consisting of (from left to right) a positive control well, a negative control well, a single specimen well and lastly a duplicate positive control well. To simulate the variability inherent in point-of-care testing, specimens were processed at the shelters where they were obtained or at Purdue Veterinary Medicine by the co-authors or either of two laboratory technicians ('field method') rather than by a single individual. In addition to the manufacturer's recommended interpretation of test results as 'positive' or 'negative,' results were reported using a semi-quantitative evaluation scheme as follows:

Negative results:

- NegNCV, no color visible.
- NegVSC, very slight color but obviously less than positive control.
- NegCCV, considerable color but clearly less than positive control.
- NegSPC, color appears to be similar but not equivalent to positive control.

Positive results:

- PosEPC – appears to be equivalent to positive control.
- PosMMPC – marginally more color than positive control.
- PosSMPC – significantly more color than positive control.

Aliquots of sera from a subset of dogs from the Humane Society of Indianapolis were also processed using a modified method, whereby extra wash steps were added. In the 'extra wash' method, six individual washes (vs. three washes recommended by the manufacturer) were used for each of the two wash steps.

At the time that the tests were performed, personnel were masked to the results of the 'gold standard' CPV HI and CDV SN tests.

'Gold standard' measurement of CPV HI titers and CDV SN titers

The SN test for CDV was done as previously described by Appel and Robson (1973) using Vero cells and the Onderstepoort strain of CDV. In brief, sera were tested in duplicate in 96-well microtitre plates with microscopic detection of viral cytopathology after a 5 day incubation period. Antibody titer (reciprocal of the dilution at the end point) calculations were based on serum dilutions (initial serum dilution of 1:4) and 50% end point determinations.

Antibody titers for CPV-2 were determined by HI assays as previously described by Carmichael et al. (1980). All sera were adsorbed with a 50% suspension of swine red blood cells to remove non-specific inhibitors. Initial serum dilution for the HI test was 1:10.

Statistical methods

Sensitivity and specificity for dichotomous data (positive/negative test results) were calculated using Win Episcope 2.0.[2] Agreement of categorical data (semi-quantitative evaluation scheme) was calculated using simple linear regression after checking residual plots for normality (StatsDirect statistical software Version 2.7.8). Data were transformed for CPV HI titers by calculating the log to base 2 of 0.1× the original titer and for CDV SN titers by calculating the log to base 2 of 0.25× the original titer prior to linear regression.

Results

A total of 200 serum samples were collected from 108 dogs, including 93 sera from PAWS Chicago (Day 1, $n = 51$; Days 6–8, $n = 30$; Days 13–15, $n = 12$) and 107 sera from the Humane Society of Indianapolis (Day 1, $n = 57$; Days 6–8, $n = 32$; Days 13–15, $n = 18$). Results reported as either 'positive' or 'negative' were compared against the CPV HI titers and CDV SN titers to generate sensitivity and specificity data (Tables 1 and 2). Table 3 reports the results of linear regression performed to test agreement between semi-quantitative results and logarithmically transformed 'gold standard' results. All correlation coefficients (r) values were significantly different from zero ($P < 0.0001$).

There were a number of discordances when results obtained using the regular method and reported as either 'positive' or 'negative' were compared against the gold standard CPV HI titers and CDV SN titers. For CPV, both specimens that yielded false positive results ($n = 2/199$; 1.0%) had CPV HI titers within one dilution of the cut-off titer for protection (antibody titer = 80). For CDV, 6/7 false positive specimens ($n = 7/200$; 3.5%) had CDV SN titers within one dilution of the cut-off titer used for protection by either the ELISA test kit manufacturer (antibody titer = 16) or the reference laboratory (antibody titer = 32). For false negative results, 7/13 discordant CPV results ($n = 13/199$; 6.5%) were within one dilution of the cut-off titer and 7/28 discordant CDV results ($n = 28/200$; 14.0%) were within one dilution of one of the two cut-off points. Using the regular method, the true prevalence of a positive CPV result was 84.4% (95% confidence intervals 79.4, 89.5) and it was 57.5% for a positive CDV result (95% confidence intervals 50.6, 64.4). Modification of the manufacturer's recommended protocol via three extra washes minimally improved accuracy, sensitivity, and specificity of the point-of-care test for detection of either serum CPV or CDV antibodies (Tables 1–3).

Discussion

The sensitivity and specificity of the ELISA test when performed as a point-of-care test according to the manufacturer's instructions under field conditions exceeded 90% except for CDV protective antibody titer sensitivity, which was 75.7% (67.8%, 83.5%). In general, the diagnostic accuracy for CPV was better than for CDV,

[1] Synbiotics TiterCHEK CDV/CPV direction insert – http://www.synbiotics.com/Products/CompanionAnimals/Canine/TiterCHEK-CDV-CPV-Parvo/96-0460-DI.pdf (last accessed 06.06.11).

[2] http://www.clive.ed.ac.uk/winepiscope/.