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Dot-blot ELISA assay demonstrates strong agreement with hemagglutination inhibition for the detection of canine parvovirus antibodies in sera of healthy, vaccinated blood-donor dogs

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Objective
To compare canine parvovirus (CPV) antibody titers in sera of community blood-donor dogs using the hemagglutination inhibition (HI) assays from 2 state veterinary diagnostic laboratories (VDLs) and a dot-blot ELISA assay to determine intermethod agreement.

Methods
Stored sera (–80-°C freezer) from 100 healthy community-based blood-donor dogs were selected. Dogs represented various breeds and ages. Available vaccination history was retrospectively assessed. Sera were thawed and delivered to the VDLs, with HI and the dot-blot ELISA assay performed the following day.

Results
Of 100 samples, 96 dot-blot ELISA results were within a 2-fold dilution of the corresponding HI titer from the Colorado State University VDL, and 92 were within a 2-fold dilution of the HI titer from the New York State VDL. The dot-blot ELISA assay demonstrated high sensitivity (96% and 97%) and strong agreement (Spearman ρ = 0.72 and 0.92). Of 43 dogs with vaccination history, 93% had protective titers. The 3 lowest titers occurred in dogs vaccinated within 1 year.

Conclusions
CPV antibody titers in community blood-donor dogs showed strong agreement between HI assays and the dot-blot ELISA assay. A high concordance and strong correlation support the dot-blot ELISA assay as a reliable method for estimating CPV antibody titers.

Clinical Relevance
Accurate, rapid assessment of CPV titers is important for selecting plasma from community blood-donor dogs intended for passive immunotherapy in critically ill parvoviral patients. Demonstration of strong agreement between the dot-blot ELISA and reference HI assays supports the use of this assay for in-house donor screening and timely plasma selection.

Keywords: parvovirus, canine, antibody, plasma, hemagglutination inhibition

Canine parvovirus (CPV) is a life-threatening virus found worldwide, typically infecting puppies and young vaccination-naïve dogs with incomplete immunity.[1] It is a single-stranded DNA virus that replicates in the nucleus of rapidly dividing cells of the lymphoid tissue, small intestinal epithelium, and bone marrow, with continued systemic circulation during infection (viremia).[1] If left untreated, the combination of decreased immunity and bacteremia secondary to translocation from the gastrointestinal tract puts these patients at high risk of developing septic shock, systemic inflammatory response syndrome, multiorgan dysfunction, or death.[1] In cases of severe damage to the gastrointestinal epithelium, there are significant losses of albumin and other proteins, resulting in hypovolemia and systemic cardiovascular instability. Plasma (frozen or fresh frozen) is a commonly used natural oncotic blood product that is obtained through appropriately vaccinated and screened dogs from community blood banks.[2] Plasma products are usually administered to provide oncotic support in dogs with severe CPV infection; however, plasma products may also provide passive immunotherapy

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due to the presence of CPV-specific antibodies, support the endothelial glycocalyx, and offer other natural substances such as fibronectin that lessen bacteremia.[3,4] While it could be beneficial to know which plasma products have the highest CPV antibody titers prior to administration, most veterinary blood banks do not currently provide that information.

A commonly utilized option to determine CPV antibody titers is hemagglutination inhibition (HI) assays, which are typically performed at state veterinary diagnostic laboratories or other commercial laboratories. While some laboratories use standardized procedures for serologic testing, results may still vary between diagnostic laboratories, highlighting the importance of evaluating intermethod and interlaboratory agreement when comparing antibody titers.[5,6] Another limitation to the use of HI for CPV titer determination is the need for sample shipping, which leads to additional expense and negates the ability to determine CPV titers in individual plasma units in real time, which may be pertinent in an emergency setting.

A rapid canine distemper (CDV), CPV, and canine adenoviral (CAV) dot-blot ELISA test kit (VacciCheck; BioGal Galed Labs) is approved for use in some countries and is typically marketed to identify protective serum antibodies for repeated vaccination requirements.[7–9] To our knowledge, the kit has not been evaluated for quantifying CPV antibody titers in samples from community blood-donor dogs. Therefore, the primary objective of this study was to compare CPV titers obtained using a dot-blot ELISA assay with those determined by HI assays performed at 2 state veterinary diagnostic laboratories to assess intermethod agreement. The secondary and final objectives were to evaluate variability in CPV titer results when the same sample was assessed by multiple assays and to explore associations between time since last vaccination and CPV antibody titers. We hypothesized that the dot-blot ELISA would demonstrate strong agreement with reference HI assays and that most dogs vaccinated against CPV within 3 years would have a positive titer of ≥ 1:80, consistent with protective immunity.[10–12]

Methods

Case selection

Convenience samples of stored sera were selected from 100 healthy screened dogs participating in a community-based blood-donor program at the Colorado State University Veterinary Teaching Hospital Blood Bank Service. Serum was used for all analyses because HI assays are validated and routinely performed using serum, and archived serum samples were available from routine donor screening. This ensured methodological consistency across all assays. The only requirement for the study was that a minimum of 500 µL of sera was available to meet the total quantitative needs of the 3 assays to be used. Prior consent to participate in the blood donation program constituted informed owner consent for the study; therefore, specific IACUC approval to use these remnant samples was not required. The dogs had been preselected and screened to ensure suitability as donors per recent guidelines and represented various breeds and ages.[2] The most recent CPV booster vaccination was retrospectively retrieved when available via medical record review, email, and telephoning of owners.

Sample storage and handling

Sera had been stored in a –80-°C freezer since the last health screening of the dogs via the Blood Bank. Each sample was thawed at room temperature, and 2 aliquots were made under aseptic conditions in order to meet the minimum requirements for the HI assays provided by the 2 veterinary diagnostic laboratories (Colorado State University Veterinary Diagnostic Laboratory [100 µL] and New York State Veterinary Diagnostic Laboratory [200 µL]). The samples were shipped on cold packs to New York by overnight express and stored at 4 °C at Colorado State University. The 2 HI assays and the dot-blot ELISA assay were all performed on the same day. The HI assays were performed with the standard operating procedures of the 2 state laboratories. Similar to other HI studies comparing titers within and between laboratories, titers are considered concordant if they varied by no more than 1 dilution higher or lower than the comparative titer (2-fold).[13–15]

Dot-blot ELISA assay

The dot-blot ELISA assay contained specific antigens for CAV, CPV, and CDV fixed onto a thin plastic comb tooth surface, with 12 comb teeth to a testing panel (*comb*). The assay provided semiquantitative results, reported as CombScale scores (S0 to S6), which corresponded to manufacturer-defined HI titer ranges. Upon arrival at Colorado State University, the kit was stored in a refrigerator at 2 to 8 °C (35 to 46 °F) until used. The dot-blot ELISA assay was performed and interpreted by the same investigator (CTT) on the same day, who was blinded to both the vaccination history and HI results of each sample.

On the day the assays were completed, the developing plate was brought up to room temperature (20 to 25 °C; 68 to 77 °F) by incubating at 37 °C (98.6 °F) for 25 minutes. The plate had 6 rows, each with 12 wells, labeled A to F that contained wash buffer, conjugate, or chromogen that was covered by a thin aluminum casing. The developing plate was gently mixed by rocking back and forth several times prior to use, with the row labeled A subsequently pierced to expose the underlying well. Five microliters of sera from each sample was placed within a respective A row well and mixed with the pipette 5 times by raising and lowering the plunger of the pipette. The comb was removed from a protective envelope, and each of the 12 teeth was placed within the allocated row A well and left to incubate for 5 minutes. During the 5 minutes, the comb was moved up and down in the well 4 times, avoiding contact of the front surface of the tooth with the edge of the well. This process was repeated 4 times at the 4:00, 3:00, 2:00, and 1:00 intervals of the 5-minute countdown. Nonsterile tweezers provided by the manufacturer were used to pierce the row B wells. The comb was removed from row A, with

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excess liquid tapped off 5 times onto a paper towel prior to placing into row B for 2 minutes. During the 2 minutes, the comb was moved up and down in the well 4 times, avoiding contact of the front surface of the tooth with the edge of the well. This process was repeated 4 times at the 1:36, 1:12, 0:48, and 0:24 intervals of the 2-minute countdown. This process was repeated for each of the subsequent rows, with mixing of the teeth per a 5-minute or 2-minute protocol. At the final incubation step, the comb teeth exhibited a gray-scalar color change **(Figure 1)**.

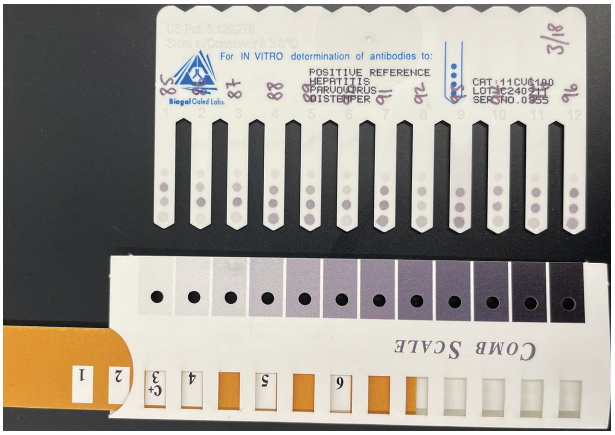

Figure 1—Representative completed dot-blot ELISA assay (VacciCheck; BioGal Galed Labs) comb (top) with the manufacturer-provided gray-scalar reference (bottom). Each comb tooth contains 4 vertically aligned reaction spots. From bottom to top, the spots correspond to canine distemper virus antigen, canine parvovirus antigen, canine adenovirus antigen, and an internal positive control. Development of grayscale color at the antigen spots indicates the presence of virus-specific IgG antibodies, while the control spot confirms assay validity for each tooth.

A manufacturer-provided grayscale reference was used to assign the CombScale score that most closely matched the observed color intensity. Per manufacturer data, these scores corresponded to approximate HI titer ranges as follows: S0 to S1, negative; S2, 1:40; S3, 1:80; S4, 1:160; S5, 1:320; S6, ≥ 1:640. These equivalents were used to facilitate comparison with HI assay results while recognizing the semiquantitative nature of the dot-blot ELISA assay. Any CPV test result spot that did not yield a gray color at the corresponding CPV site was considered negative.

Statistical methods

Descriptive statistics were calculated with commercially available software (Excel; Microsoft Corp). The sampled population consisted entirely of healthy, previously vaccinated blood-donor dogs with an expected high prevalence of detectable antibody titers. Therefore, the data set lacked a true unexposed or seronegative control group. Only sensitivity was calculated, as specificity and

Table 3—Diagnostic agreement between a commercial dot-blot ELISA CPV antibody titer assay and HI assays from 2 state diagnostic laboratories.

	Colorado State	New York State
True positive	96	92
True negative	0	1
False positive	0	4
False negative	4	3
Sensitivity (%)	96	97
False-negative rate (%)	4	3

False positives indicate dot-blot ELISA titers higher than HI results; false negatives indicate dot-blot ELISA titers lower than HI results.

Table 1—Cross-tabulation of canine parvovirus (CPV) antibody titers determined by commercial dot-blot ELISA assay versus state diagnostic laboratory (Colorado State University) hemagglutination inhibition (HI) assay.

	HI titer										
Dot-blot ELISA	**Neg**	**16**	**32**	**64**	**128**	**256**	**512**	**1,024**	**2,048**	**4,096**	**8,192**
Neg	—	—	—	—	—	1	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—	—	—
40	—	—	—	—	—	—	—	—	—	—	—
80	—	—	1	—	—	—	—	—	—	—	—
160	—	—	—	1	4	3	2	—	—	—	—
320	—	—	—	—	3	11	8	1	—	—	—
≥ 640	—	—	—	—	—	—	13	30	18	4	—

— = Not applicable.

Table 2—Cross-tabulation of CPV antibody titers determined by commercial dot-blot ELISA assay versus state diagnostic laboratory (New York State) HI assay.

	HI titer										
Dot-blot ELISA	**Neg**	**10**	**40**	**80**	**160**	**320**	**640**	**1,280**	**2,560**	**5,120**	**10,240**
Neg	1	—	—	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—	—	—
40	—	—	—	—	—	—	—	—	—	—	—
80	—	1	—	—	—	—	—	—	—	—	—
160	—	—	1	—	7	2	—	—	—	—	—
320	—	—	—	2	3	13	2	1	—	2	—
≥ 640	—	—	—	—	—	1	16	19	19	8	2

— = Not applicable.

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Table 4—Distribution of CPV antibody titers determined by the New York state HI assay and time since last known vaccination.

Time since vaccination	Total dogs	Titer magnitude										
		Neg	10	40	80	160	320	640	1,280	2,560	5,120	10,240
Within 1 y	25	1	1	1	1	1	4	4	3	7	1	1
Within 2 y	10	0	0	0	0	1	1	2	4	1	1	0
> 2 y	8	0	0	0	0	2	0	2	3	0	1	0

false-positive rate could not be meaningfully derived in the absence of known negative samples. Spearman rank correlation coefficient (ρ) were calculated with commercially available software (Excel; Microsoft Corp) and was used to measure the strength of the relationship between the results from each laboratory and the dot-blot ELISA assay. A ρ value of > 0.7 was considered a strong correlation. A titer of ≥ 1:80 in the HI was used to denote a protective titer in the comparisons with time between vaccination and testing.

Results

A total of 100 canine sera samples were included in this study. The dot-blot ELISA assay CPV titers ranged from 1:40 to ≥ 1:640. Canine parvovirus titers from the New York State Laboratory ranged from < 1:10 to 1:10,240, while the Colorado State University Veterinary Diagnostic Laboratory CPV titers ranged from 1:32 to 1:4,096. As the Colorado State HI titers did not align numerically to the New York State and dot-blot ELISA assay, an equivocal range was identified and used accordingly (ie, 1:10 was equivocal to 1:16, 1:40 was equivocal to 1:32, 1:80 was equivocal to 1:64, etc). Ninety-six and 92 dot-blot ELISA assay results were within a 2-fold dilution of the corresponding state veterinary diagnostic HI titer and were therefore considered concordant **(Tables 1 and 2)**, including 1 sample that was negative by both the dot-blot ELISA and HI assays. Four dot-blot ELISA results exceeded the HI titer by more than 2-fold, and 7 were more than 2-fold lower, resulting in 11 nonconcordant samples. These discrepancies would not have altered the clinical interpretation, as the majority (8 of 11 [72%]) fell within a detectable and protective (≥ 1:80) antibody titer range. The dot-blot ELISA assay demonstrated strong performance in detecting dogs with measurable antibody titers, with high sensitivity of 96% and 97%, and strong agreement (ρ = 0.75 and 0.92) between both the Colorado State and New York State veterinary diagnostic laboratories, respectively **(Table 3)**.

Of the 43 dogs with available vaccination history, all were vaccinated within 4.5 years, and 40 (93%) had positive titers suggesting protection. The 3 dogs with the lowest CPV titers had been vaccinated within 1 year **(Table 4)**. The vaccination products used were unknown.

Discussion

The current study evaluated agreement amongst estimated titers from a commercially available dot-blot ELISA CPV antibody assay and titers determined by HI performed at 2 state veterinary diagnostic laboratories. Our results demonstrated strong agreement (ρ = 0.75 and 0.92) as well as excellent sensitivity (96% and 97%), supporting the dot-blot ELISA assay as a rapid tool for accurately identifying plasma donors with detectable CPV antibodies.

The rapid identification of plasma donors with adequate CPV antibodies is critical for veterinary blood banks and clinicians, in particular those in emergency and critical care settings who actively manage severely affected naïve or unvaccinated patients. Passive immunotherapy using CPV antibody containing plasma has emerged as a potential adjunctive treatment for CPV enteritis, aiming to provide immediate immunological support while minimizing gastrointestinal clinical signs and decreasing mortality.[16–20] However, the ability to select plasma units with demonstratable CPV antibody titers is essential to maximize therapeutic efficacy in these situations. More recently, CPV monoclonal antibody (CPMA) therapy has been shown to prevent mortality following experimental challenge, with evidence that CPMA has a higher affinity for CPV-2 than native IgG, resulting in potent viral neutralization.[20,21] While the CPMA represents a distinct and promising intervention, plasma-based passive immunotherapy remains widely accessible and clinically relevant, reinforcing the importance of reliable donor antibody screening. Conventional HI testing is considered the gold standard; however, it requires submission to external state veterinary diagnostic laboratories that are capable of performing this unique diagnostic test. This process results in logistical delays and additional costs, which may limit its feasibility for routine blood-donor screening. In this study, the dot-blot ELISA assay has shown its ability to provide a convenient alternative to the current external send-out protocol, enabling a rapid point-of-care assessment of CPV antibody status, with results known within minutes instead of days.

The strong correlation between the dot-blot ELISA assay's semiquantitative scores and HI titers further supports its validity as a surrogate measure of accurate CPV antibody level reporting. Although the dot-blot ELISA assay maximum score of 4+ corresponds to a titer of ≥ 1:640, several blood-donor sera in this cohort had HI titers exceeding this level (Tables 1 and 2), reaching a maximum of 1:10,240 per external state laboratory HI findings. While the range of the dot-blot ELISA assay is limited compared to the state laboratory HI testing, its intended use in this study was to accurately identify the agreement between its quantitative titer and the gold standard HI results of 2 state veterinary diagnostic laboratories. For screening purposes on the clinical floor, particularly in the context of blood-donor programs, a binary assessment of presence of high antibody titer (≥ 1:640)

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versus absence would be considered sufficient and highly valuable. However, future work will need to be performed to determine the CPV virus neutralization capability of samples using different HI or dot-blot ELISA assay titers.

Our findings aligned with recent research that has evaluated the same dot-blot ELISA assay for CPV, CAV, and CDV antibody detection.[9,22,23] These studies identified adequate detection of protective vaccinal antibody for CPV, CAV, and CDV when using the dot-blot ELISA assay and comparing their results to serum neutralization or HI testing in a healthy vaccinated dog population.[9,18,19] Unfortunately, in these studies the dot-blot ELISA assay results are reported only as a CombScale (S-number) index value to indicate protective vaccination status, without conversion to manufacturer-suggested HI titer equivalents or quantitative comparison with reference assays. Our study was able to build upon these previous studies by assessing the assay's quantitative performance in a clinically applicable cohort of blood donors whose plasma was intended for clinical use. Additionally, we compared the results of the dot-blot ELISA assay to 2 separate reference laboratories with differing methodologies and titer thresholds, providing a robust, real-world challenge across a range of laboratory conditions. The consistent performance of the dot-blot ELISA assay across both of these laboratories reinforces the assay's reliability for in-house clinical adoption and application.

Using the results from the New York State Laboratory HI assay, protective titers (≥ 1:80) were detected in 97 of 100 dogs overall. When analysis was limited to the 43 dogs with known vaccination history, 40 (93%) had titers consistent with protection. Similar findings were identified with the Colorado State University HI assay, in which 98 of 100 dogs had titers ≥ 1:80. These results are consistent with prior studies.[10–12] The 3 dogs with the lowest CPV titers were all reportedly vaccinated within 1 year. It is unlikely that this finding reflected delayed antibody development and more likely that it represented individual variability in vaccine response. Whether the low or negative titers were vaccine product related could not be determined. Detailed age data were not available for all dogs, preventing assessment of age-related factors such as immunosenescence or young adult hyporesponsiveness and genetic nonresponder status. In addition, it is unknown whether the owner or a veterinary healthcare provider provided the CPV vaccination, and it is possible that an inferior product and/or an inappropriately handled product was administered. Alternately, most dogs vaccinated 2 or 3 years previously had detectable titers, including a dog vaccinated 4.5 years earlier that had an estimated titer of 1:640. It is difficult to assess the maximal duration of vaccine-associated titers using samples from client-owned animals in the field since the individual dogs may have been unknowingly boosted by exposure to other dogs administered modified-live CPV containing vaccines or by exposure to field strains of CPV. These findings emphasize that routine CPV antibody titer testing may be particularly valuable in dogs enrolled in blood-donor programs, as vaccine-associated variability in antibody magnitude can result in substantial differences between donors. The identification of donors with consistently high CPV titers would allow blood banks to characterize and label plasma units accordingly, potentially increasing their therapeutic value. From a clinical perspective, the ability to preferentially select high titer plasma units at the time of presentation may enhance the effectiveness of passive immunotherapy, reduce delays in treatment, and support more targeted use of blood products in emergency and critical care settings.

Limitations of this study included the retrospective sample collection from a convenience population of healthy, appropriately vaccinated blood-donor dogs. Due to the population from which these sera samples were obtained, it remains unknown whether the dot-blot ELISA assay would perform similarly in other populations such as naturally infected CPV dogs, dogs that have a waning immunity, or those that are immunocompromised. Future prospective studies would be valuable to evaluate whether rapid identification and administration of high–CPV antibody titer plasma is associated with decreased disease severity and improved clinical outcomes in dogs with parvovirus enteritis. Such studies would focus on donor plasma characterization rather than diagnostic testing of affected dogs, as the dot-blot ELISA detects IgG rather than IgM and is not intended to guide diagnosis or acute treatment decisions in symptomatic patients. In addition, this study evaluated antibody titers in serum rather than plasma. Although antibody concentrations are expected to correlate, this relationship was not directly assessed and represents an important limitation. Another limitation of this study was its focus on sensitivity and correlation rather than a full diagnostic accuracy analysis, including specificity, accuracy, and predictive values. While our findings suggested a low rate of false positives and false negatives (overestimate and underestimate of titers, respectively), formal assessment of specificity would require a larger cohort with a population of dogs that have not received routine vaccinations or that have been exposed to CPV. However, the clinical priority for plasma donor screening is highly sensitive to avoid missing high-titer units, and the dot-blot ELISA assay appears to meet this need effectively. Finally, the dot-blot ELISA assay had a maximum titer limit of 1:640, while the HI veterinary diagnostic state laboratory assays detected titers up to 1:10,240. This underestimated a number of very high titers as detected by the HI assay. However, this is unlikely to affect the binary clinical decision when identifying plasma units with high CPV antibody titers, particularly when higher CombScale scores (S5 or greater) are preferentially selected. Because this study population lacked a true seronegative control group, false-positive rates could not be formally assessed, and misclassification could theoretically reduce the effectiveness of passive immunotherapy if plasma were assumed to contain CPV antibodies or not. This risk may be mitigated by prioritizing donors with high semiquantitative scores when available. In addition, the dot-blot ELISA detects IgG

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only, whereas HI assays detect antibody classes additively, including IgM, which may partially account for differences observed between assays. Future considerations to modifying and expanding the assay's dynamic range, allowing for the semiquantitative distinction between very high and moderate antibody titers, could further enhance its utility for research and clinical applications.

The findings from this study were clinically significant. This study has demonstrated that the dot-blot ELISA assay enables a rapid, affordable, reliable, and decentralized tool for screening blood-donor dogs for appropriate CPV antibodies within their plasma. This method of detection effectively removes the barriers associated with external laboratory-based HI testing. Both academic and private practice blood banks now have the ability to rapidly identify plasma units without waiting for HI results to return for a patient that may benefit from high–CPV antibody plasma for passive immunotherapy. Emergency and critical care veterinarians may also benefit from immediate confirmation of antibody status when planning patient treatments, specifically those cases with severe CPV infection where time-sensitive interventions can impact clinical outcomes.

In conclusion, CPV antibody titers in community-based blood-donor dogs showed strong agreement between both state veterinary diagnostic laboratory HI tests and a commercial dot-blot ELISA assay. A high level of concordance was observed, with 97% and 96% of the samples within a 2-fold dilution of the corresponding HI titer, respectively, and strong agreement. Although minor discrepancies in titer estimation were noted, the dot-blot ELISA assay was shown to reflect antibody concentrations in a real-time clinical setting, supporting its potential utility in screening donor plasma units on the clinical floor and in blood-banking environments.

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Disclosures

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