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Sensitivity & Specificity of ELISA Kit for Antibody Detection of Canine Parvovirus and Canine Distemper Infection in Dogs

Hassenin ASH[1,*], Aneela Durrani[2]

[1]*Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Egypt*

[2]*Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Lahore, Pakistan*

ABSTRACT

Canine parvovirus (CPV) and canine distemper virus (CDV) are the common fetal disease of canine. Their control and prevention of these diseases include vaccination and high care should be done is important in canine shelters throughout the world. ELISA is a sero-chemical reaction for the helps for the detection of protective. Antibodies of these against both above viruses. The study helps to identify the goal was to confirm the specificity and sensitivity of ELISA and in comparison, compare it with with CPV hemagglutination inhibition and CDV serum neutralization test. s using sera collected from dogs housed at animal shelters. The ELISA was used under both field and laboratory conditions and duplicate specimens were processed using an extra wash step. The accuracy of test kits in serum samples results showed that the sensitivity of CDV was 94.0%, using an optical density meter 88.1% with a specificity of 91.8%. For CPV, the sensitivity was 92.3% with 93.5% specificity. This test appears to be a good indicator for antibody detection against both infections.

Keywords: ELISA, Titers, Virus, Canine, Sensitivity, Specificity.

INTRODUCTIONS

The most common highly fatal contagious diseases wide spreading all over the world of domesticated canines called canine parvovirus (CPV) and canine distemper virus (CDV) [1-4]. Although vaccination is available for those viral infections still big concerns with high mortality and morbidity in unvaccinated pets in shelters, shops, and puppy mills [1,2,5]. Immuno-response and antibody protection against CPV and CDV remarkable and increasing titers in serum following vaccination for solid immunity in susceptible dogs [6]. The titer of antibodies in serum should be measured for detecting the need for vaccination in regular and routine time [7], for extra precaution and protection in domesticated and sheltered 28 canines [8,9].

Challenging antibody titers for immunity and protection considering for virulence of viral strain and correct dose size compared to the sufficiency of immune-mediated cytotoxicity for production of the memory cell for

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***Corresponding Author**

Hassenin ASH

Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Egypt, Tel: +1 6127706945

Email: amirasaad@zu.edu.eg; amm_microhass@yahoo.com.

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prevent further infection and Forming T-helper cell-mediated immunity for different levels of antibody concentration in serum for further and long protection. Laboratory and field tests for accurate immunity detection from viral infection in domesticated and shelters dog during outbreaks recommend serological tests as well as chemical kits to give more antibody detection with different titers level at variable protection times for best results with minimal risk [10].

The serological test used for accurate detection level of antibody titers in serum for CPV were hemagglutination inhibition (HI), virus neutralization (VN) tests, indirect fluorescent antibody assays (IFA), and recently by ELISA.

Following vaccination titer of antibodies in serum with adequate amount was measured by HI ≥1:80 [11,12]. CDV titers are measured using ELISA, IFA, and serum neutralization (SN) tests [7]. Early CDV challenge studies using SN determined that titers of 1:30-1:100 were protective [13,14], while a later study reported that titers of ≥1:32 (equivalent to an IFA result ≥1:5) indicated a sufficient antibody response to vaccination [12].The Synbiotics Titer CHEK CDV/CPV test1 is a point-of-care ELISA kit marketed for the rapid determination of protective serum antibody concentrations in dogs against CPV and CDV [15].

RESULTS

are interpreted as 'positive' or 'negative' for each virus, with the package insert claiming that a positive result for 49 CPV indicates an antibody titer equivalent to a CPV HI titer ≥1:80 and a positive for CDV indicates an antibody titer equivalent to a CDV SN titer ≥1:16. The present study aimed 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

Sample collection

Blood samples were collected from dogs admitted at veterinary hospital, faculty of veterinary medicine, Zagazig University, Egypt, with different ages showing clinical signs of infection and asymptomatic healthy ones. All blood samples were transferred to the lab for serum separation and refrigerated for serological diagnosis. A target enrolment of 65 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. Dog: n = 13

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 Abbasyia: Pfizer Duramune Max 5; dog clinical infection: Pfizer Vanguard Plus 5). All dogs that were suspected not to have protective titers against both CPV and CDV by the Titer CHEK CDV/CPV test kit performed on day 1 were retested on days 6-8. Dogs that might not have had protective titers against both CPV and CDV at the day 6-8 recheck were retested from 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 (Zagazig University Animal Health Diagnostic Center) and (2) submission to the Synbiotics Corporation for Titer Check testing under laboratory conditions by one laboratory technician and by a microplate reader (ELx800 Universal Microplate Reader, Bio Tek Instruments).

Checkpoint of ELISA

The ELISA (Synbiotics Titer CHEK CDV/CPV test) was used according to the manufacturer's instructions, reporting results as either positive or negative. 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 Zagazig Veterinary Medicine by the 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. Negative results were categorized as follows: (1) NegNCV, no color visible; (2) NegVSC, very slight color but less than positive control; (3) NegCCV, considerable color but less than positive control; and (4) NegSPC, color appears to be similar but not equivalent to positive control. The positive results were differentiated as (1) osEPC, which appears to 86 be equivalent to the positive control; (2) PosMMPC, marginally more color than the positive control; and (3) PosSMPC, significantly more color than the positive control.

Aliquots of sera from a subset of dogs from animal hospitals, clinics 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 reference.

Measurement standard Titers of CDV SN & CPV HI

CPV HI titers

The SN test for CDV was done as previously described by Appel and Robson [16], using Vero cells and the Onderstepoort strain of CDV. 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 (see Appendix A: Supplementary material).

CPV HI titers

Antibody titers against CPV-2 were determined by HI assays as described by Carmichael et al. [17]. All sera were adsorbed with a 50% suspension of porcine red blood cells to remove non-specific inhibitors. The initial serum dilution for the HI test was 1:10.

Data analysis

Sensitivity and specificity for dichotomous data (positive/ negative test results) were calculated using commercially available software. Agreement of categorical data (semi-quantitative evaluation scheme) was calculated using simple linear regression after checking residual plots for normality (Stats Direct statistical software Version 2.7.8). Data were transformed for CPV HI titers by calculating the log2 of 0.1x the original titer and, for CDV SN titers, by calculating the log2 of 0.25x the original titer before linear regression.

RESULTS AND DISCUSSION

A total of 156 serum samples were collected from 78 dogs (Apparent healthy: n = 65; Diseased Dog: n = 13). Among the study population, there were 69 Native -breed dogs, 16 imported strains, and 53 (0-6 months) young puppets with 77adult above 1 years Ninety-three specimens were collected from dogs admitted to animal hospital (day 1, n = 51; days 6-8, n = 30; days 13-15, n = 12) and 107 specimens from dogs at clinic (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 a linear regression performed to test the agreement between semi-quantitative results and logarithmically transformed reference standard results. All correlation coefficients (r) values were significantly different from zero (P <0.0001).

Table 1. Validation of specificity and sensitivity of hemagglutination inhibition (HI) for titers detection against canine parvovirus (CPV) antibody titers

	Sensitivity % (96% CI)	Specificity % (96% CI)
Testing check point related to manufacturer- protocol used (*n* = 199)	93.2 (87.2-94.3)	94.5 (85.8-99.8)
Check point with extra dilution [a](*n* = 102)	93.4(88.2-99.1)	94.1 (81.9-99.9)
Referenced laboratory-performed kits used Protocol recommendation (*n* = 138)	93.8 (91.0-98.9)	88.9 (74.4-100.0)
Optical density (OD) measurement (*n* = 138)	94.2 (88.7-97.8)	89.9 (74.4-100.0)

96% CI, 96% confidence interval.

a In the 'extra wash' method, six individual washes (vs. three washes recommended by the manufacturer) were used for each of the two wash steps.

Table 2. Validation of specificity and sensitivity of ELISA for the detection of serum canine distemper virus (CDV) antibody titers

	Sensitivity % (96% CI)	Specificity % (96% CI)
Testing check point related to manufacturer- protocol protocol (*n* = 156)	74.8 (66.9-82.7)	92.3 (84.7-96.8)
Check point with extra dilution [a](*n* = 87)	77.2 (66.2-86.8)	92.7 (85.0-99.8)
Referenced laboratory-performed kits used Protocol recommendation (*n* = 138)	93.8 (87.4-98.8)	84.7 (76.8-93.9)
Optical density (OD) measurement (*n* = 117)	87.8 (81.2, 96.7)	88.9 (87.9, 95.8)

96% CI, 96% confidence interval.

a In the 'extra wash' method, six individual washes (vs. three washes recommended by the manufacturer) were used for each of extra dilution steps).

There were several discordances when results obtained using the regular method and reported either 'positive' or 'negative' were compared against the reference 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 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 seven of 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 133 prevalence of CPV was 84.4% (95% confidence intervals 79.4-89.5) and it was 57.5% for CDV (95% confidence intervals 50.6-64.4). Modification of the manufacturer's recommended protocol via three extra washes minimally improved the accuracy, sensitivity, and specificity of the point-of-care 136 test for the detection of either serum CPV or CDV antibodies (Tables 1-3).

Table 3. Semi-quantitative evaluation scheme results of ELISA antibody test against canine parvovirus (CPV) hemagglutination inhibition titers/canine distemper (CDV) serum neutralization titers performed at a reference laboratory

	CPV r^2 (n)	CDV r^2 (n)
Elisa antobody titers	0.8 (106)	0.9 (156)
HI antibody titers	0.64 (67)	0.73 (86)
SN antibodies titers	0.56 (77)	0.72 (54)
Synbiotics Titer Check	0.51 (45)	0.63 (66)

The sensitivity and specificity of the ELISA 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% (95% confidence interval 67.8-83.5%). In general, the diagnostic accuracy for CPV was better than for CDV, although a comparison of the 95% confidence intervals reveals that there is overlap when test results from the same methodology are compared between viruses, except for the sensitivity results for the regular method and the extra wash method, both performed under field conditions (Tables 1 & 2). While this reduction in the ability to detect an animal with a positive CDV titer could lead to a decision to administer vaccination unnecessarily, this is far preferable to using a test with relatively poor specificity, which could result in exposing a susceptible animal to infection. This is particularly important in a shelter, where infection is more likely because of the combination of an increased environmental viral load and a population of animals with varying health status and vaccination histories [9].

There was some discordance between serum ELISA results obtained using the regular method and reference standard

CPV HI and CDV SN results. False positive ELISA results could occasionally occur when non-neutralizing antibodies result in a positive point-of-care test result but are ineffective at HI or SN. False negatives could occur because of low ELISA sensitivity, particularly when low HI or SN antibody concentrations are induced by vaccination and yet are sufficient to induce protection from challenge. We presume that this is the reason why some dogs might have had undetectable or otherwise 'negative' results when tested by the ELISA at the 6-8 or 13-15 day time points, as this length of time should have been sufficient for seroconversion, antibody maturation, and isotype switching to have occurred.

Field use of point-of-care tests requires that diagnostic accuracy remains high under highly variable conditions. To simulate these conditions and evaluate their effect, the ELISA results from three locations (both animal shelters and Purdue Veterinary Medicine), were compared with those obtained in a laboratory with a single technician. For CPV, point-of-care testing resulted in similar sensitivity, but superior specificity, to laboratory-performed testing, whereas for CDV point-of-care testing produced inferior sensitivity but superior specificity to laboratory testing (Tables 1 & 2). It is possible that these were chance occurrences associated with poor statistical power and inadequate sample size rather than real findings since a review of the 95% confidence intervals reveals overlap for all except the CDV sensitivity results and the confidence intervals were relatively wide.

When the laboratory-performed test results were compared with the optical density meter results from the same laboratory, the results were very similar, leading to the conclusion that human eyes can discriminate color differences as accurately as calibrated optical devices and that subjective color assessment is not a major source of error when performing this ELISA. Similarly, the semiquantitative method produced very good levels of agreement with the reference standard results (Table 3), but it appears unlikely that the semi-quantitative methodology appreciably improved diagnostic accuracy when compared with results reported as either 'positive' or 'negative', as the manufacturers recommend. The results for the regular method and the extra wash method were remarkably similar. This is most likely because the number of washes in the regular method was enough to rid the wells of unbound reagents so that they did not subsequently interfere with the binding of antibodies or conjugate during the assay [18-20].

CONCLUSIONS

A Checkpoint of antibody titers against CDV/CPV using ELISA is a useful tool to in-clinic to determine CDV and CPV antibody in a dog. Status when used according to manufacturer's instructions under field conditions and could be it is useful used in any dog population to help control to control the outbreak of disease. A disease outbreak, however a decline in Low serum protective antibodies to below those levels in the serum is considered to be protective is not synonymous with more vulnerability to infection; A long-term protection (i.e. years beyond vaccination) from CDV or CPV infection is likely to persist due to long-lived undifferentiated T memory cells, CD4+ T-helper cells (i.e. cell-mediated immunity) and CD8+ T-cells (i.e. cytotoxic T cells). S As such, the test is most suitable for determining for vaccination and knows the protective level for the disease. Point-of-care identification of dogs that do not require vaccination; negative results are most likely to be sensitive, but not specific, for identifying those dogs vulnerable to CDV or CPV infection.

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CONFLICT OF INTEREST STATEMENT

Synbiotics Corporation supplied the ELISA kits used in this study. Corporations played no role in the study design nor the collection, analysis, and interpretation of data, or in the decision to submit the manuscript for publication. None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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