RESEARCH ARTICLE

Comparison of Lateral Flow Assay and RT-PCR for Detection of *Canine Distemper Virus* in Dogs

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Abstract

The present study was focused on comparing lateral flow assay (LFA) and one-step RT-PCR for detection of *Canine distemper virus* (*CDV*) in dogs. Total of 74 swab (38 nasal and 36 conjunctival) samples from 23 CD suspected dogs were collected. Detection of *CDV* was performed by LFA using quickVET Rapid test kit and molecular detection by one-step RT-PCR targeting N gene. Based on the test result, sensitivity and specificity were calculated. The prevalence of *CD* detected by LFA and RT-PCR were 69.56% and 73.91% respectively among dogs.. All the dogs positive for *CDV* by LFA were also positive by RT-PCR, including one more dog. Comparative analysis of RT-PCR and LFA using sample-wise positivity revealed that relative sensitivity and specificity of LFA considering RT-PCR as the Gold standard test were 66.66% and 100%, while overall agreement between the two assays was 77.03%. For diagnosis of CD in dogs, RT-PCR proved to be a better test than LFA. However, the inherent advantages associated with field tests like LFA and its considerable sensitivity and specificity can be useful and adopted for field-level diagnosis.

Keywords: Canine Distemper virus, Canine distemper, Dogs, LFA, RT-PCR.

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Introduction

Canine distemper (CD) is a highly infectious, frequently a lethal disease in dogs and has a high mortality rate after Rabies (Latha *et al*., 2007). The natural host range comprises predominantly carnivores (Beineke *et al*., 2009) and domestic dogs act as ideal reservoirs of the disease (Vanak *et al*., 2007). *Canine distemper virus* (*CDV*) is a single-stranded, non-segmented, negative-sense RNA virus belonging to the genus *Morbillivirus* of family *Paramyxoviridae* of order *Mononegavirales* (MacLachlan *et al*., 2011). CD remains a significant cause of morbidity and mortality in shelters because of the presence of frequently susceptible dogs and puppies. Infection can be unapparent, mild, or severe, leading to death up to 50% of the infected animals (Newbury *et al.,* 2009). Non-vaccinated pet puppies and dogs are at high risk and *CDV* infection usually occurs in winter season (Desai *et al*., 2021). Dogs infected with virulent *CDV* strains showed obvious clinical signs of CD including conjunctivitis, ocular discharge, nasal discharge, anorexia, hyperkeratosis of digital cushions, catarrhal inflammation of bronchi and larynx, and intense pustules on the abdomen and thigh (Carvalho *et al.,* 2012). Point of care-based diagnostic aids like lateral flow assay are much important at field level for early diagnosis (Desai *et al*., 2020[b]). Since early detection of CD would allow appropriate treatment quickly, such a test helps in reducing the morbidity and mortality associated with CD to other animals (Dong *et al.,* 2008). Molecular based techniques like Reverse transcriptase PCR (RT-PCR) can be used for the confirmatory diagnosis by targeting N gene of *CDV* (Kim *et al*., 2001; Desai *et al*., 2021), but it requires costly machine, skills and longer period of time (Desai *et al*., 2020[a]). However, both techniques have their own suitable use at different diagnosis level. Considering the importance of *CDV* for canine health management and the need to study suitable diagnostic assays for prompt diagnosis, the present study was focused on comparing lateral flow assay and one-step RT-PCR for detection of *CDV* in dogs.

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Materials and Methods

Collection of Serum Samples

The study was carried out from September 2019 to February 2020. Total 74 clinical samples (38 nasal and 36 conjunctival swabs) were collected aseptically using a sterile swab from 23 dogs suspected with CD from Anand, Vadodara and Ahmedabad districts of Gujarat. The swab samples were collected from dogs at different periodic intervals within the same day. Samples were transferred to sterile disposable plastic specimen vial containing RNAlater immediately after collection and stored in deep fridge (-80°C) until further process.

Detection of *CDV* by Lateral Flow Assay

The LFA quickVET *Canine Distemper Virus* antigen (*CDV*-Ag) rapid test kit was procured from UBIO Biotechnology Systems Pvt. Ltd. (Cochin, Kerala). QuickVET *CDV*-Ag rapid test is a qualitative immunochromatographic assay for rapidly detecting *CDV*-Ag from nasal and conjunctival secretions of dogs. Test was performed according to the manufacturer's protocol.

Detection of *CDV* by RT-PCR

In the present study, total 74 swab samples were included for the *CDV* extraction. In addition, CD vaccine (DHPPi, Canigen) and a nasal swab sample of CD vaccinated apparently healthy dog were used as positive and negative control, respectively. Viral RNA was extracted using the commercially available QIAGEN QIAamp® Viral RNA Mini kit (Qiagen Sciences, MD, USA, Cat. No.52904) as per manufacturer's instructions. Viral RNA purity and quantification were done using Nanodrop ND-1000 UV-Vis Spectrophotometer (Thermofisher). The pure quality RNA (3.0 µL) was further used for RT-PCR by using SuperScript III One-step RT-PCR system with Platinum Taq DNA Polymerase Kit System (Invitrogen). The reaction mixture was made as per the kit protocol. The primers targeting the 'N' gene of CDV were used as per the Yong *et al*. (2001). One step RT-PCR cyclic conditions were included one cycle of 55°C-30 min and 94°C-two min followed by 40 cycles of 94°C-15 sec, 44°C-30 sec, 68°C-60 sec and one cycle of final extension of 68°C-5 min. Analysis of PCR products was done by agarose gel electrophoresis using 1.5 % agarose. The amplified product was envisioned as a single compact band of expected size under UV light under gel documentation system (SynGene, Gene Genius Bioimaging System, UK).

Table 1: Location, sex and age-wise prevalence of CDV in dogs

Factor	*Particulars*	*% Positive (Nos) by LFA*	*% Positive (Nos) by RT-PCR*
Location/ City	Anand (12)	66.66 (8)	66.66 (8)
	Ahmedabad (9)	77.77 (7)	88.88 (8)
	Vadodara (2)	50.00 (1)	50.00 (1)
Sex	Male (21)	66.66 (14)	71.42 (15)
	Female (2)	100.00 (2)	100.00 (2)
Age	>6-12 Month (16)	93.75 (15)	100.00 (16)
	>12-24 Month (7)	14.28 (1)	14.28 (1)
Total	Dogs (23)	69.56 (16)	73.91 (17)

Comparative Analysis

Comparative analysis of diagnostic tests, LFA and one-step RT-PCR for *CDV* detection was done by calculating sensitivity and specificity parameters by using MedCalc online server (MedCalc Software Ltd., Version 20.027; accessed January 23, 2022).

Results and Discussion

The present study assessed the incidence of CDV in dogs by quickVET *CDV*-Ag rapid test kit after collecting 74 swab samples (including 38 nasal and 36 conjunctival) of 23 dogs. Of these, 12 dogs (29 swab samples) belonged to Anand, 9 dogs (38 swab samples) belonged to Ahmedabad and the remaining 2 dogs (7 swab samples) belonged to Vadodara district. Of the total 23 dogs screened, 16 dogs were found positive by quickVET rapid test kit. The incidence was thus, determined to be 69.56%. The details of the results are given in Tables 1 and 2. The presence of *CDV* in dogs was determined by observing the presence of control as well as test band using LFA kit (Fig. 1). Almost similar incidence rate was reported in dogs by Wang *et al*. (2018), *i.e.,* 62.00%. The incidence recorded in this study was lower (77.77%) than reported by Candela *et al*. (2019), while Soma *et al.* (2003), Naghibi *et al.* (2012), Dongre *et al.* (2013), Fischer *et al*. (2013), Ogbu *et al*. (2017) and Awad (2019) recorded 26.9%, 2.82%, 9.00%, 56.60%, 45.33% and 45.28% incidence, respectively

Location-wise, out of three districts included in the study, the incidence of *CDV* by LFA was 66.66%, 77.77% and 50.00% in Anand, Ahmedabad and Vadodara districts, respectively (Table 1). In contrast, Pranitha *et al.* (2022) reported 24.24% and 6.66% from Anand and Ahmedabad districts. Swab

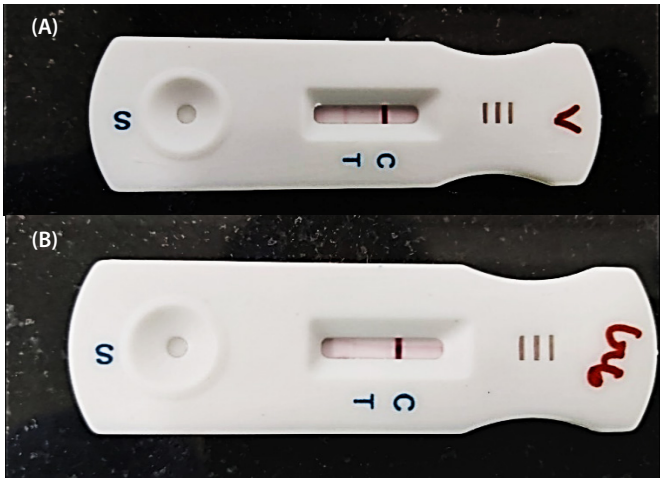

Fig.1: Immuno-chromatography based LFA test, (A) Positive result (B) Negative result

Table 2: Result of CDV swab samples in dogs by LFA and one-step RT-PCR

Sr. No.	Location	No. of swabs collected			No. of Positive swabs by LFA			No. of Positive swabs by one-step RT-PCR		
		Nasal swabs	Conjunctival swabs	Total	Nasal swabs	Conjunctival swabs	Total	Nasal swabs	Conjunctival Swabs	Total
1.	Anand	14	15	29	9	11	20	13	14	27
2.	Ahmedabad	20	18	38	9	3	12	14	8	22
3.	Vadodara	4	3	7	-	2	2	-	2	2
Total		38	36	74	18 (47.36%)	16(44.44%)	34 (45.94%)	27(71.05%)	24(66.67%)	51 (68.91%)

samples-wise positivity of *CDV* indicated that 34 out of 74 (45.94%) swab samples were found positive by LFA (Table 2). Of which, 20 positive swabs (9 nasal and 11 conjunctival) were from Anand; 12 positive swabs (9 nasal and 3 conjunctival) were from Ahmedabad and 2 positive swabs (conjunctival) were from Vadodara district. Total of 18 (47.36%) nasal swab samples were positive by LFA, while 16 (44.44%) conjunctival swab samples were found positive by LFA. The lower result of Vadodara district does not reflect valid indication due to the extremely smaller sample size available.

Results of sex-wise and age-wise prevalence of CDV by LFA presented in Table 1 revealed that 14 out of 21 male dogs and both the female dogs tested positive for the presence of *CDV* antigen. The peak incidence of *CDV* by LFA recorded was much higher in dogs of >6-12 months of age (93.75%, 15/16), than in >12-24 months of age (14.28%, 1/7). This variation was attributed to the availability of the majority of the samples from younger age group.

In the present study, nasal and conjunctival swab samples processed for *CDV* by targeting N gene segment yielded the products of 549 bp in case of positive samples, confirming the presence of *CDV* (Fig. 2). Out of the total 23 dogs screened, 17 dogs were positive for *CDV* by N gene-based RT-PCR (Table 1). The maximum incidence of *CDV* detected by RT-PCR was found in Ahmedabad district (88.88%), followed by Anand district (66.66%) and Vadodara district (50.00%). In comparison, Pranitha *et al.* (2022) reported CDV incidence 17.14% in Anand and 6.66% in Ahmedabad. When compared for *CDV* incidence detected by LFA and RT-PCR, out of 23 dogs tested, 16 (69.56%) and 17 (73.91%) were found positive. Thus, RT-PCR could detect one dog more as positive for *CDV*. Desai *et al*. (2021) also reported RT-PCR is more efficient in detecting CDV than LFA. The present finding was following Desai *et al*. (2021), where they have found 14 dogs (77.77%) positive out of 18 from the south Gujarat region. All the dogs positive for *CDV* by LFA were also positive by RT-PCR, while one dog found positive by RT-PCR was negative by LFA.

Swab samples-wise positivity of *CDV* indicated that 51 (68.91%) out of the total 74 swab samples were found positive by RT-PCR (Table 2). Of which, 27 positive swabs (13 nasal and 14 conjunctival) were from Anand; 22 positive swabs (14 nasal and 8 conjunctival) were from Ahmedabad and 2 positive swabs (conjunctival) were from Vadodara district. Out of 38 nasal swab samples, 27 (71.05%) were detected positive for *CDV* by RT-PCR. Out of 36 conjunctival swab samples, 24 (66.67%) were detected positive for *CDV* by RT-PCR (Table 2). Therefore, collecting more swab samples from the same dog improved the chances of *CDV* detection.

Results of sex-wise and age-wise prevalence of CDV by RT-PCR presented in Table 1 revealed that 15 out of 21 male dogs and both the female dogs tested positive. The incidence of *CDV* by RT-PCR recorded was much higher in dogs of >6-12 months of age (100%, 16/16), than in >12-24 months of age (14.28%, 1/7). This variation was attributed

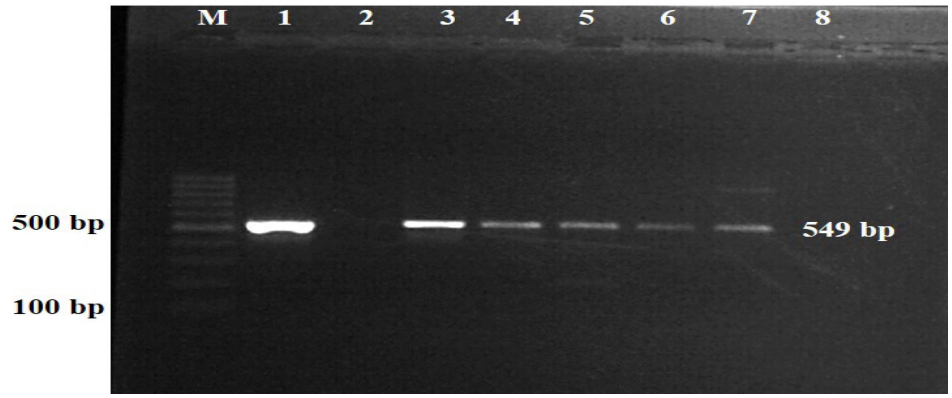

Fig. 2: RT-PCR results using swab samples for CD diagnosis. M: 100 bp ladder, 1: Positive control, 2: Negative control, 3-8: Clinical samples

Table 3: Comparative analysis of swab samples by LFA and one-step RT-PCR for CDV detection

		RT-PCR					
Test		*Positive*	*Negative*	*Total*	*Sensitivity (%)*	*Specificity (%)*	*Overall agreement (%)*
LFA	Positive	(a)34	(c)0	(a+c)34	66.66	100.00	77.03
	Negative	(b)17	(d)23	(b+d)40			
	Total	(a+b)51	(c+d)23	(c+d)74			

a= True Positive, b= False Negative, c= False Positive, d= True Negative

to the availability of majority of the samples from younger age group.

Considering one-step RT-PCR as a reference test, relative sensitivity and specificity of LFA were calculated. Comparative analysis was carried out on the swab samples and the result is presented in Table 3. Out of 74 swab samples, 34 and 51 swab samples were positive by LFA and RT-PCR, respectively, whereas 40 samples were negative by LFA and 23 swab samples were negative by RT-PCR. Seventeen samples negative by LFA were positive by RT-PCR, while none of the sample negative by RT-PCR was positive by LFA. Thus, the relative sensitivity and specificity of LFA to one-step RT-PCR were 66.66 % and 100 %, respectively. Overall agreement between the two assays was 77.03%. In a similar study using LFA for detection of *CDV*, An *et al*. (2008) reported 100 % sensitivity and specificity of LFA relative to nested PCR and indicated that conjunctival swab specimens are the most suitable specimens for early antemortem diagnosis of CD. However, they also reported that when blood lymphocytes and nasal samples were tested, the LFA assay was slightly less sensitive (89.7% and 85.7%, respectively) and specific (94.6% and 100%, respectively) than nested PCR.

Conclusions

The present study was conducted to determine the incidence of CDV and diagnostic efficacy of two different tests, LFA and RT-PCR, where the former test is rapid and point of care and the latter is the molecular-based confirmatory diagnostic test. Out of 23 dogs, 16 (69.56%) dogs were found positive by LFA and 17 (73.91%) dogs were found positive by RT-PCR. Although RT-PCR was more efficient than LFA for *CDV* diagnosis, inherent advantages associated with field-side test like LFA showing considerable sensitivity and specificity can be useful for field-level diagnosis of canine distemper.

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ANNOUNCEMENT: SVSBT-NS-2022

IX Annual Convention and National Seminar of SVSBT

The ***IX Annual Convention*** and ***National Seminar*** of The Society for Veterinary Science & Biotechnology ***(SVSBT) on "Recent Biotechnological Advances in Health and Management to Augment Productivity of Livestock and Poultry"*** will be **organized at Ramayanpatti, Tirunelveli - 627 358, Tamil Nadu, during September 22-24, 2022** (Thursday, Friday & Saturday) by Veterinary College & Research Institute, Tirunelveli - 627 358, TANUVAS, (TN). The detailed Brochure cum Invitation showing Theme Areas/ Sessions, Registration Fee, Bank Details for online payment and deadlines, etc. has been floated on the Whats Apps and e-mails. Accordingly, the organizing committee of ***SVSBT NS-2022 invites abstracts*** of original and quality research work on theme areas of seminar limited to 250 words by e-mail on svsbttnns2022@gmail.com or mopandian69@gmail.com latest by 30th August, 2022 for inclusion in the Souvenir cum Compendium to be published on the occasion.

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