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Short communication

Establishment of reverse transcription loop-mediated isothermal amplification for rapid detection and differentiation of canine distemper virus infected and vaccinated animals



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ABSTRACT

Although widespread vaccination against canine distemper virus (CDV) has been conducted for many decades, several canine distemper outbreaks in vaccinated animals have been reported frequently. In order to detect and differentiate the wild-type and vaccine strains of the CDV from the vaccinated animals, a novel reverse transcription loop-mediated isothermal amplification (RT-LAMP) method was developed. A set of four primers—two internal and two external—were designed to target the H gene for the specific detection of wild-type CDV variants. The CDV-H RT-LAMP assay rapidly amplified the target gene, within 60 min, using a water bath held at a constant temperature of 65 °C. The assay was 100-fold more sensitive than conventional RT-PCR, with a detection limit of 10⁻¹ TCID₅₀ ml⁻¹. The system showed a preference for wild-type CDV, and exhibited less sensitivity to canine parvovirus, canine adenovirus type 1 and type 2, canine coronavirus, and canine parainfluenza virus. The assay was validated using 102 clinical samples obtained from vaccinated dog farms, and the results were comparable to a multiplex nested RT-PCR assay. The specific CDV-H RT-LAMP assay provides a simple, rapid, and sensitive tool for the detection of canines infected with wild-type CDV from canines vaccinated with attenuated vaccine.

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1. Introduction

Canine distemper (CD) is a highly contagious and fatal disease of dogs and other terrestrial carnivores. CD is caused by the canine distemper virus (CDV), a single-stranded negative RNA virus of the genus *Morbillivirus*, family *Paramyxoviridae*, and is closely related to the viruses responsible for measles, rinderpest, peste des petits ruminants, phocine distemper, dolphin distemper, porpoise distemper, and equine morbillivirus (Fenner, 1976; Haas and Barrett, 1996; McCarthy et al., 2011; Soltan and Abd-Eldaim, 2014; Bellière et al., 2011).

It has been widely documented that CDV causes severe immunosuppression and persistent infection in the central nervous system in dogs (Müller et al., 1995; Wu et al., 2000; Meertens et al., 2003; Vandeveld and Zurbriggen, 2005). Moreover, the host spectrum of CDV is gradually increasing (Qiu et al., 2011). Currently, vaccination is the most effective means of controlling CD and one of the most frequently used vaccines is a modified live attenuated vaccine. Although widespread vaccination against CD has been conducted for many decades, this infection still represents an important disease for dogs (Elia et al., 2006). There is, therefore, a need for the development of assays that can distinguish dogs vaccinated with modified live attenuated vaccine from those infected with wild-type virus in herds.

Rapid and sensitive detection methods, including the reverse transcription polymerase chain reaction (RT-PCR) and multiplex PCR, are currently available for the diagnosis of CDV. However, the occurrence of false-positive PCR products for each of these methods hinders the identification of wild-type CDV strains (Ralston et al., 2007). Real-time quantitative PCR methods have

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also been reported to detect CDV. The advantages of real-time PCR, compared with conventional RT-PCR, include higher speed, greater sensitivity, and less handling of PCR products. However, real-time PCR instruments are expensive and may not be readily available in many laboratories (Coleman et al., 2014). Other methods such as serological assays (Indirect immunofluorescence assay (IFA), and Enzyme linked immunosorbent assay), virus isolation and in situ hybridization have also been developed, but are not applicable for the fast and sensitive diagnosis of CDV (Gray et al., 2012; Gaedke et al., 1997; Rzezutka and Mizak, 2002; Frisk et al., 1999; von Messling et al., 1999).

Loop-mediated isothermal amplification (LAMP) is a novel method for the rapid, specific, and sensitive amplification of nucleic acids (Notomi et al., 2000). With its advantages, it has been applied widely in the detection of pathogenic microorganisms (Dinh et al., 2011; Li and Cai, 2011; Njiru et al., 2012) and genetically modified foods (Fukuta et al., 2004), analysis of shelf-life in melon (Fukuta et al., 2006) and chromosomal chimerism of bovine (Hirayama et al., 2007).

Rapid and cost-effective RT-LAMP assays for the pre-clinical detection of CDV have been described elsewhere (Cho and Park, 2005; Liu et al., 2010; Wen et al., 2012; Hu et al., 2012). As these assays target both wild-type CDV variants and the live attenuated vaccine strain, the interpretation of the results may be complicated in terms of eradication and control where a vaccination policy is practiced.

This study aimed to establish a RT-LAMP method which was specific for wild-type CDV, could differentiate from canines vaccinated with attenuated CDV vaccine, and which could be applied with relative ease in minimally-equipped laboratories and under field conditions.

2. Materials and methods

2.1. Viruses and field samples

CDV strains: wild-type CDV YB strain (Asia-1 subgroup), LDH (06) strain (Asia-1 subgroup), and ZH (05) strain (Asia-2 subgroup) were isolated by using Madin–Darby Canine Kidney expressed SLAM of dog (MDCK-SLAM) cells, and were identified and maintained at the Harbin Veterinary Research Institute (Harbin, China). Three locally supplied CDV vaccine products Vacc-A (Intervet, Netherlands: Onderstepoort strain), Vacc-B (Pfizer, USA: Snyder Hill strain) and Vacc-C (Merial, France: RECOMBITEK C4) were tested in the current study. All other non-CDV canine viruses: canine parvovirus (CPV), canine adenovirus type 1 and type 2 (CAV-1 and CAV-2), canine coronavirus (CCoV), and canine parainfluenza virus (CPIV) were provided by the Harbin Veterinary Research Institute (Harbin, China). Field samples ($n = 102$; including 37 rectal swabs, 20 conjunctival swabs, 15 urine samples, and 30 whole blood samples) (Table 1) were collected from different farms across several provinces of China where vaccination with live attenuated vaccine was in operation.

2.2. Ethical considerations

This study was approved by the Harbin Veterinary Research Institute Experimental Animal Welfare Ethics Committee (HVRI-EAWEC), and conducted under the guidance of the HVRI-EAWEC. The animals from which specimens were collected were handled in accordance with animal protection law of the People's Republic of China. Our sampling processes were assisted by local authorities and veterinarians. All the owners of the dogs gave permission for their animals' samples to be used in this study.

Table 1

The information of the clinical samples collected from domestic dogs in 2012.

Location (Province)	Specimens	Numbers	Clinical signs
Heilongjiang	Rectal swabs	15	Fever, sneeze, depression, cough, diarrhea or bloody stool
	Conjunctival swabs	13	
	Urine	5	
	Whole blood	13	
Jilin	Rectal swabs	10	Spasm, fever, depression, sneeze and cough
	Urine	4	
	Whole blood	10	
Liaoning	Rectal swabs	12	Depression and diarrhea
	Whole blood	7	
Shandong	Conjunctival swabs	7	Sneeze, cough, spasm and fever
	Urine samples	6	

2.3. Primer design

Genomic sequences of N and H gene were aligned using the MegAlign program of DNASTar software (DNASTar, Madison, WI, USA). With online LAMP designing software PrimerExplorer V4 (<http://primerexplorer.jp/e/>), two sets primers (F3-N and B3-N, FIP-N and BIP-N) and (F3-H and B3-H, FIP-H and BIP-H) were designed based on the wild-type CDV YB strain (Table 2). The former was common for all types of CDV strains; the latter was conserved among wild-type CDV strains but not for the live attenuated CDV vaccine strains.

2.4. RNA extraction and cDNA synthesis

Viral RNA was extracted using the QIAamp Viral RNA Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Reverse transcription was performed in a final reaction volume of 20 μ l containing: 10 μ l RNA, 4 μ l 5 \times RT Buffer, 1 μ l dNTP (10 mM each), 50 pmol 9-Random Hexamers, 10 U M-MLV reverse transcriptase (Takara, Dalian, China), and 20 U RNase inhibitor (Takara, Dalian, China). The reaction was incubated at 42 $^{\circ}$ C for 1 h followed by incubation at 70 $^{\circ}$ C for 10 min.

2.5. The universal CDV-N RT-LAMP assay for detection of CDV

The CDV-N RT-LAMP assay was performed in a total volume of 25 μ l consisting of: 0.3 μ M F3-N and B3-N primers, 2.4 μ M FIP-N and BIP-N primers, 1.4 mM dNTPs, 2.5 μ l 1 \times Thermo Pol buffer, 8 U Bst DNA polymerase (New England BioLabs, Herts, UK), and 2 μ l cDNA. The reaction mixture was incubated in a water bath for 45–50 min at 65 $^{\circ}$ C prior to incubation at 80 $^{\circ}$ C for 2 min. 6 μ l of the LAMP products were subjected to electrophoresis on a 2%(w/v) agarose gel.

2.6. The specific CDV-H RT-LAMP assay for detection of wild-type CDV

The CDV-H RT-LAMP assay was performed in a total volume of 25 μ l consisting of: 0.2 μ M F3-H and B3-H primers, 1.2 μ M FIP-H and BIP-H primers, 1.4 mM dNTPs, 2.5 μ l 1 \times Thermo Pol buffer, 12 U Bst DNA polymerase (New England BioLabs, Herts, UK) and 2 μ l cDNA. The reaction condition was same as above.

2.7. Evaluation of the specificity, sensitivity and reproducibility of CDV-H RT-LAMP assay

Specificity of the CDV-H RT-LAMP assay for wild-type CDV was assessed against CDV vaccine strains (Vacc-A, Vacc-B and Vacc-C),

Table 2
Details of RT-LAMP and multiplex nested RT-PCR primers for CDV.

Primers ^a	Type	Sequences
F3-N	Forward outer primer	5'-GCAAAGTAAGCTCGGCACTT-3'
B3-N	Reverse outer primer	5'-TGGTTTTCGGACCTCTTGT-3'
FIP-N	Forward inner primer	5'-CGGTCTCCGTTGTCTTGGATGGCTTGGCATCACCAAGGAA-3'
BIP-N	Reverse inner primer	5'-CGCTGCTGGTCCCAAGCAATGGGGTTGTTGATTAGCGACT-3'
F3-H	Forward outer primer	5'-GCCTGTATTGGTCTCTGAG-3'
B3-H	Reverse outer primer	5'-CTCCG TTCAGTATAACCGG-3'
FIP-H	Forward inner primer	5'-TGCACATAGGGTAGGATTTTCTGAACAAGAGGAGCAAAAAAAGT-3'
BIP-H	Reverse inner primer	5'-AGTTGCTTCTTATGGGCGGATGTTAAGTTGAAGTCAATGC-3'
CDV-P1	F (For CDV species)	5'-AAATCCTGTGTACCCGCTC-3'
CDV-P2	F (For wild-type strain)	5'-TGGTGGCTCTGCAATATGAA-3'
CDV-P3	F (For vaccine strain)	5'-AATGAATGGATGCTCGGGTTT-3'
CDV-P4	R(For CDV species)	5'-ACGTCCTGGACCCTAAGTTT-3'

^a The primers of F3-N, B3-N, FIP-N, BIP-N, F3-H, B3-H, FIP-H, BIP-H were for RT-LAMP Primers. CDV-P1, CDV-P2, CDV-P3 and CDV-P4 were applied in multiplex nested RT-PCR.

CDV wild-type isolates (ZH (05) and LDH (06)), and non-CDV canine viruses (CPV, CAV-1, CAV-2, CPIV, and CCoV). PCR products amplified from the CDV-YB strain with two external primers F3 and B3 were cloned into pMD18-T Vector (Takara, Dalian, China) and sequenced to validate the results of CDV-H RT-LAMP assay. To establish the sensitivity of the CDV-H RT-LAMP and RT-PCR assay with the primers of CDV-P1/CDV-P4, CDV-YB viral stocks obtained from infected cells were titrated, their titers determined, 10-fold serial dilutions prepared, and viral RNA extracted from 140 μ l per dilution. Inter- and intra-assay reproducibility tests were performed in triplicate by testing four cell cultures infected with the CDV-YB strain against four mock-infected cells (data not shown).

2.8. Comparison of the CDV-H RT-LAMP and a multiplex nested RT-PCR assay for detection of CDV in field samples

Field samples ($n = 102$) from different dog farms in China were tested in parallel with both the CDV-H RT-LAMP assay and a multiplex nested RT-PCR assay (Si et al., 2010).

2.9. Virus isolation

Virus isolation of field samples was performed by using MDCK-SLAM cells (Seki et al., 2003). At least three passages in cell cultures were conducted, followed by IFA using the antisera for CDV.

3. Results

3.1. Performance of the CDV-N RT-LAMP assay

With regard to the dilution series for the CDV-YB strain, the CDV-N RT-LAMP assay detected a limit of 10^{-1} TCID₅₀ ml⁻¹ of CDV, as observed by gel electrophoresis (Fig. 1A). The assay detected three strains of wild-type CDV and three vaccine strains, as demonstrated by the bands of differing sizes visualized by agarose electrophoresis, which is consistent with the presence of several inverted-repeat structures for RT-LAMP products (Fig. 1B). No RT-LAMP products were detected for CPV, CAV-1, CAV-2, CPIV and CCoV (Fig. 1C).

3.2. Performance of the CDV-H RT-LAMP assay

With regard to the dilution series for the CDV-YB strain, the CDV-H RT-LAMP assay detected a limit of 10^{-1} TCID₅₀ ml⁻¹ of CDV, and was 100-fold more sensitive than conventional RT-PCR, as observed by gel electrophoresis (Fig. 2A and B). The assay detected two strains of wild-type CDV, as demonstrated by the

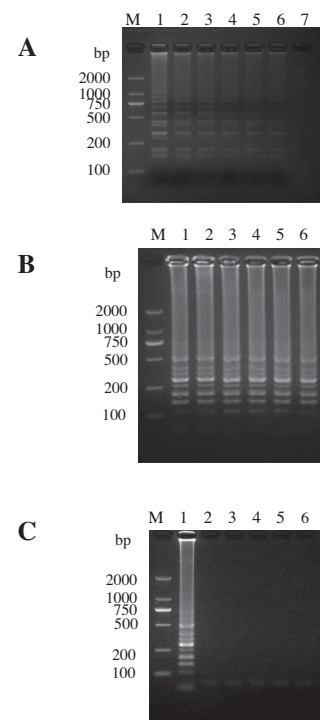


Fig. 1. Sensitivity and specificity of the CDV-N RT-LAMP assay. (A) Sensitivity of the CDV-N RT-LAMP assay. M: DL2000 DNA marker; 1–7: cDNA from different titers of CDV-YB: 10^4 TCID₅₀ ml⁻¹ (1), 10^3 TCID₅₀ ml⁻¹ (2), 10^2 TCID₅₀ ml⁻¹ (3), 10^1 TCID₅₀ ml⁻¹ (4), 10^0 TCID₅₀ ml⁻¹ (5), 10^{-1} TCID₅₀ ml⁻¹ (6), and 10^{-2} TCID₅₀ ml⁻¹ (7). (B and C) Specificity of the CDV-N RT-LAMP assay. (B) M: DL2000 DNA marker; 1–6: ZH (05), YB, LDH (06), Vacc-A, Vacc-B and Vacc-C. (C) M: DL2000 DNA marker; 1–6: CDV-YB, CPV, CAV-1, CAV-2, CCoV and CPIV.

bands of differing sizes visualized by agarose electrophoresis, which is consistent with the presence of several inverted-repeat structures for RT-LAMP products (Fig. 2C). No RT-LAMP products were detected for CPV, CAV-1, CAV-2, CPIV and CCoV (Fig. 2C). Similar results were obtained from three independent tests of four positive and negative samples, performed in triplicate for the inter- and intra-assays, and which demonstrated the reproducibility of the assay (results not included).

3.3. Comparison of the CDV-H RT-LAMP assay with a multiplex nested RT-PCR assay and virus isolation for detection of CDV

Of the 102 field samples, 13 were positive for CDV using both assays. The remaining 89 samples were negative for CDV when

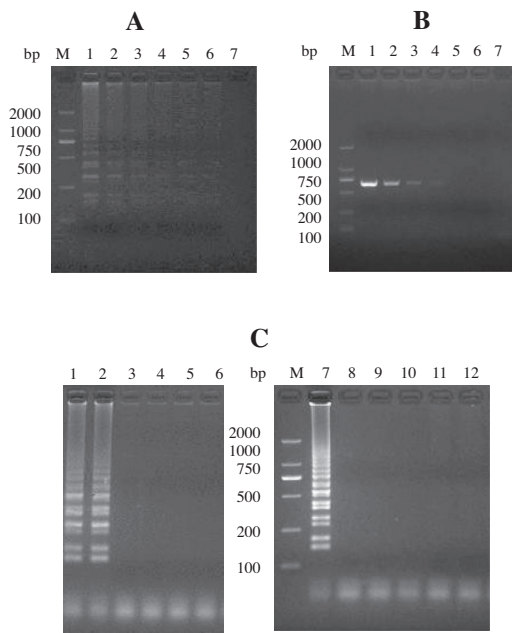


Fig. 2. Sensitivity and specificity of the CDV-H RT-LAMP assay. (A and B) Sensitivity of the CDV-H RT-LAMP assay and RT-PCR (CDV-P1/CDV-P4). M, DL2000 DNA marker; 1–7, cDNA from different titers of CDV-YB: 10^4 TCID₅₀ ml⁻¹ (1), 10^3 TCID₅₀ ml⁻¹ (2), 10^2 TCID₅₀ ml⁻¹ (3), 10^1 TCID₅₀ ml⁻¹ (4), 10^0 TCID₅₀ ml⁻¹ (5), 10^{-1} TCID₅₀ ml⁻¹ (6) and 10^{-2} TCID₅₀ ml⁻¹ (7). (C) Specificity of the CDV-H RT-LAMP assay. M: DL2000 DNA markers; 1: ZH (05); 2: LDH (06); 3–6: Vacc-A, Vacc-B, Vacc-C and Negative control; 7–12: CDV-YB, CPV, CAV-1, CAV-2, CCoV and CPiV.

tested with the RT-LAMP assay, while 87 samples were classified as negative for the multiplex nested RT-PCR assay. The result of CDV-H RT-LAMP assay was consistent with the virus isolation, which is the “gold standard” for the detection of CDV.

4. Discussion

Canine distemper virus is the etiological agent for a serious and often fatal disease in dogs and many other carnivores. CDV is responsible for several fatal outbreaks in wild carnivores, including the decimation of almost an entire population of African wild dogs (*Lycaon pictus*) in a Tanzanian reserve, the deaths of approximately 10,000 Caspian seals (*Phoca caspica*), and a loss of approximately 35% of Serengeti lions (*Panthera leo*) in an African national park (van de Bildt et al., 2002; Kuiken et al., 2006; Roelke-Parker et al., 1996).

The control of canine distemper can realistically only be achieved through vaccination (Chappuis, 1995). One of the currently available vaccines (Onderstepoort strain) elicits robust immunity in dogs, but is insufficiently attenuated for other species; high levels of mortality can occur because of its remaining virulence (Carpenter et al., 1976; Barrett, 1999). In general, the vaccine is safe, and highly efficacious in preventing CD in dogs (Gassen et al., 2000). However, the unsuccessful immunisation of dogs against CD has been reported in several countries (Blixenkrone-Møller et al., 1993; Patronek et al., 1995; Gemma et al., 1996; Ek-Kommonen et al., 1997; Jóźwik and Frymus, 2002).

Many PCR assays can differentiate between dogs vaccinated with modified-live attenuated vaccine from those infected with wild-type virus in herds (Wang et al., 2011; Yi et al., 2012), but RT-LAMP is easier to perform and provides more rapid results.

The success of the RT-LAMP method relies on the specificities of the designed primers for the target RNA sequence. To develop a RT-LAMP method for the specific detection of wild-type CDV

variants, we first designed specific primers by targeting the H gene. Under the reaction conditions, the amplification was completed in 45–50 min at a constant temperature of 65 °C. The results demonstrated that the RT-LAMP assay was able to differentiate wild-type CDV isolates from the vaccine strains, with a detection limit of 10^{-1} TCID₅₀ ml⁻¹ of CDV, and in the absence of cross-reactivity with CAV, CPV, CPIV and CCoV. Furthermore, 102 clinical samples obtained from vaccinated dogs were tested by both RT-LAMP and multiplex nested RT-PCR. Of the samples tested, 13 were positive for both assays. The remaining 89 samples were negative for the RT-LAMP assay, and 87 samples were classified as negative. These results indicate that the specific CDV-H RT-LAMP assay provides a simple, rapid, and sensitive tool for the detection of canines infected with wild-type CDV from canines vaccinated with attenuated vaccine, and has potential use for laboratory and clinical diagnosis practices.

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References

- Barrett, T., 1999. Morbillivirus infections, with special emphasis on morbilliviruses of carnivores. *Vet. Microbiol.* 69, 3–13.
- Bellièvre, E.N., Esperón, F., Sánchez-Vizcaíno, J.M., 2011. Genetic comparison among dolphin morbillivirus in the 1990–1992 and 2006–2008 Mediterranean outbreaks. *Infect. Genet. Evol.* 11, 1913–1920.
- Blixenkrone-Møller, M., Svansson, V., Have, P., Orvell, C., Appel, M., Pedersen, I.R., Dietz, H.H., Henriksen, P., 1993. Studies on manifestations of canine distemper virus infection in an urban dog population. *Vet. Microbiol.* 37, 163–173.
- Carpenter, J.W., Appel, M.J., Erickson, R.C., Novilla, M.N., 1976. Fatal vaccine induced canine distemper virus infection in black-footed ferrets. *J. Am. Vet. Med. Assoc.* 169, 961–964.
- Chappuis, G., 1995. Control of canine distemper. *Vet. Microbiol.* 44, 351–358.
- Cho, H.S., Park, N.Y., 2005. Detection of canine distemper virus in blood samples by reverse transcription loop-mediated isothermal amplification. *J. Vet. Med. B Infect. Dis. Vet. Public Health* 52, 410–413.
- Coleman, J.W., Wright, K.J., Wallace, O.L., Sharma, P., Arendt, H., Martinez, J., DeStefano, J., Zamb, T.P., Zhang, X., Parks, C.L., 2014. Development of a duplex real-time RT-qPCR assay to monitor genome replication, gene expression and gene insert stability during in vivo replication of a prototype live attenuated canine distemper virus vector encoding SIV gag. *J. Virol. Methods* 213C, 26–37.
- Dinh, D.T., Le, M.T., Vuong, C.D., Hasebe, F., Morita, K., 2011. An updated loop-mediated isothermal amplification method for rapid diagnosis of H5N1 avian influenza viruses. *Trop. Med. Health* 39, 3–7.
- Ek-Kommonen, C., Sihvonen, L., Pekkanen, K., Rikula, U., Nuotio, L., 1997. Outbreak of canine distemper in vaccinated dogs in Finland. *Vet. Rec.* 141, 380–383.
- Elia, G., Decaro, N., Martella, V., Cirone, F., Lucente, M.S., Lorusso, E., Di Trani, L., Buonavoglia, C., 2006. Detection of canine distemper virus in dogs by real-time RT-PCR. *J. Virol. Methods* 136, 171–176.
- Fenner, F., 1976. Paramyxoviridae. *Intervirology* 7, 59–60.
- Frisk, A.L., König, M., Moritz, A., Baumgärtner, W., 1999. Detection of canine distemper virus nucleoprotein RNA by reverse transcription-PCR using serum, whole blood, and cerebrospinal fluid from dogs with distemper. *J. Clin. Microbiol.* 37, 3634–3643.
- Fukuta, S., Mizukami, Y., Ishida, A., Kanbe, M., 2006. Development of loop-mediated isothermal amplification (LAMP)-based SNP markers for self-life in melon (*Cucumis melo* L.). *J. Appl. Genet.* 47, 303–308.
- Fukuta, S., Mizukami, Y., Ishida, A., Ueda, J., Hasegawa, M., Hayashi, I., Hashimoto, M., Kanbe, M., 2004. Real-time loop-mediated isothermal amplification for the CaMV-35S promoter as a screening method for genetically modified organisms. *Eur. Food Res. Technol.* 218, 496–500.
- Gaedke, K., Zurbriggen, A., Baumgärtner, W., 1997. In vivo and in vitro detection of canine distemper virus nucleoprotein gene with digoxigenin-labelled RNA, double-stranded DNA probes and oligonucleotides by in situ hybridization. *Zentralbl. Veterinärmed B* 44, 29–40.
- Gassen, U., Collins, F.M., Duprex, W.P., Rima, B.K., 2000. Establishment of a rescue system for canine distemper virus. *J. Virol.* 74, 10737–10744.

- Gemma, T., Watari, T., Akiyama, K., Miyashita, N., Shin, Y.S., Iwatsuki, K., Kai, C., Mikami, T., 1996. Epidemiological observations on recent outbreaks of canine distemper in Tokyo. *J. Vet. Med. Sci.* 58, 547–550.
- Gray, L.K., Crawford, P.C., Levy, J.K., Dubovi, E.J., 2012. Comparison of two assays for detection of antibodies against canine parvovirus and canine distemper virus in dogs admitted to a Florida animal shelter. *J. Am. Vet. Med. Assoc.* 240, 1084–1087.
- Haas, L., Barrett, T., 1996. Rinderpest and other animal morbillivirus infections: comparative aspects and recent developments. *J. Vet. Med.* 43, 411–420.
- Hirayama, H., Katagiri, S., Kageyama, S., Minamihashi, A., Moriyasu, S., Sawai, K., Onoe, S., Takahashi, Y., 2007. Rapid sex chromosomal chimerism analysis in heterosexual twin female calves by loop-mediated isothermal amplification. *Anim. Reprod. Sci.* 101, 38–44.
- Hu, J.X., Wen, Y.J., Huo, Z.Y., Shi, X.C., Yang, B.C., Wu, H., Gao, W.F., 2012. Establishment and preliminary application development of a loop-mediated isothermal amplification assay for detection of canine distemper virus. *Chin. Anim. Husbandry Vet. Med.* 39, 45–49.
- Jóźwik, A., Frymus, T., 2002. Natural distemper in vaccinated and unvaccinated dogs in Warsaw. *J. Vet. Med. B Infect. Dis. Vet. Public Health* 49, 413–414.
- Kuiken, T., Kennedy, S., Barrett, T., Van de Bildt, M.W., Borgsteede, F.H., Brew, S.D., Codd, G.A., Duck, C., Deaville, R., Eybatov, T., Forsyth, M.A., Foster, G., Jepson, P.D., Kydyrmanov, A., Mitrofanov, I., Ward, C.J., Wilson, S., Osterhaus, A.D., 2006. The 2000 canine distemper epidemic in Caspian seals (*Phoca caspica*): pathology and analysis of contributory factors. *Vet. Pathol.* 43, 321–338.
- Li, Y., Cai, S.H., 2011. Sensitive and rapid detection of the insect pathogenic fungus *Metarhizium anisopliae* var. *anisopliae* by loop-mediated isothermal amplification. *Curr. Microbiol.* 62, 1400–1404.
- Liu, C.Y., Wang, J.G., Yuan, D.L., Wang, S., Zhu, X.C., Liu, W.Q., 2010. Development of a reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) system for diagnosis of canine distemper virus. *Chin. Vet. Sci.* 40, 368–372.
- McCarthy, A.J., Shaw, M.A., Jepson, P.D., Brasseur, S.M., Reijnders, P.J., Goodman, S.J., 2011. Variation in European harbour seal immune response genes and susceptibility to phocine distemper virus (PDV). *Infect. Genet. Evol.* 11, 1616–1623.
- Meertens, N., Stoffel, M.H., Cherpillod, P., Wittek, R., Vandeveld, M., Zurbriggen, A., 2003. Mechanism of reduction of virus release and cell–cell fusion in persistent canine distemper virus infection. *Acta Neuropathol.* 106, 303–310.
- Müller, C.F., Fatzer, R.S., Beck, K., 1995. Studies on canine distemper virus persistence in the nervous system. *Acta Neuropathol.* 89, 438–445.
- Njiru, Z.K., Yeboah-Manu, D., Stinear, T.P., Fyfe, J.A., 2012. Rapid and sensitive detection of *Mycobacterium ulcerans* using a loop-mediated isothermal amplification test. *J. Clin. Microbiol.* 10, 1737–1741.
- Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., Hase, T., 2000. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res.* 28, E63.
- Patronek, G.J., Glickman, L.T., Johnson, R., Emerick, T.J., 1995. Canine distemper infection in pet dogs: a case control study of risk factors during a suspected outbreak in Indiana. *J. Am. Anim. Hosp. Assoc.* 31, 230–235.
- Qiu, W., Zheng, Y., Zhang, S.F., Fan, Q.S., Liu, H., Zhang, F.Q., Wang, W., Liao, G.Y., Hu, R.L., 2011. Canine distemper outbreak in rhesus monkeys, China. *Emerg. Infect. Dis.* 17, 1541–1543.
- Ralston, S.H., Afzal, M.A., Helfrich, M.H., Fraser, W.D., Gallagher, J.A., Mee, A., Rima, B., 2007. Multicenter blinded analysis of RT-PCR detection methods for paramyxoviruses in relation to Paget's disease of bone. *J. Bone Miner Res.* 22, 569–577.
- Roelke-Parker, M.E., Munson, L., Packer, C., Kock, R., Cleaveland, S., Carpenter, M., O'Brien, S.J., Pospischil, A., Hofmann-Lehmann, R., Lutz, H., Mwamengele, G.L., Mgasa, M.N., Machange, G.A., Summers, B.A., Appel, M.J., 1996. A canine distemper virus epidemic in Serengeti lions (*Panthera leo*). *Nature* 379, 441–445.
- Rzezutka, A., Mizak, B., 2002. Application of N-PCR for diagnosis of distemper in dogs and fur animals. *Vet. Microbiol.* 88, 95–103.
- Seki, F., Ono, N., Yamaguchi, R., Yanagi, Y., 2003. Efficient isolation of wild strains of canine distemper virus in Vero cells expressing canine SLAM (CD150) and their adaptability to marmoset B95a cells. *J. Virol.* 77, 9943–9950.
- Si, W., Zhou, S., Wang, Z., Cui, S.J., 2010. A multiplex reverse transcription-nested polymerase chain reaction for detection and differentiation of wild-type and vaccine strains of canine distemper virus. *Virol. J.* 7, 86.
- Soltan, M.A., Abd-Eldaim, M.M., 2014. Emergence of peste des petits ruminants virus lineage IV in Ismailia Province, Egypt. *Infect. Genet. Evol.* 28, 44–47.
- Van de Bildt, M.W., Kuiken, T., Visee, A.M., Lema, S., Fitzjohn, T.R., Osterhaus, A.D., 2002. Distemper outbreak and its effect on African wild dog conservation. *Emerg. Infect. Dis.* 8, 211–213.
- Vandeveld, M., Zurbriggen, A., 2005. Demyelination in canine distemper virus infection: a review. *Acta Neuropathol.* 109, 56–68.
- von Messling, V., Harder, T.C., Moennig, V., Rautenberg, P., Nolte, I., Haas, L., 1999. Rapid and sensitive detection of immunoglobulin M (IgM) and IgG antibodies against canine distemper virus by a new recombinant nucleocapsid protein-based enzyme-linked immunosorbent assay. *J. Clin. Microbiol.* 37, 1049–1056.
- Wang, F., Yan, X., Chai, X., Zhang, H., Zhao, J., Wen, Y., Wu, W., 2011. Differentiation of canine distemper virus isolates in fur animals from various vaccine strains by reverse transcription-polymerase chain reaction-restriction fragment length polymorphism according to phylogenetic relations in china. *Virol. J.* 27, 85.
- Wen, H., Qin, H.B., He, X.L., Zhu, Q., Gao, Y.L., Zhang, H.D., 2012. Detection of canine distemper virus by reverse transcriptase loop-mediated isothermal amplification method. *Chin. J. Comp. Med.* 22, 51–55.
- Wu, S.C., Chueh, L.L., Pang, V.F., Jeng, C.R., Lee, C.C., Liu, C.H., 2000. Pathology and RT-PCR employed in the identification of canine distemper virus infection in Taiwan. *J. Chin. Soc. Vet. Sci.* 26, 328–338.
- Yi, L., Cheng, S., Xu, H., Wang, J., Cheng, Y., Yang, S., Luo, B., 2012. Development of a combined canine distemper virus specific RT-PCR protocol for the differentiation of infected and vaccinated animals (DIVA) and genetic characterization of the hemagglutinin gene of seven Chinese strains demonstrated in dogs. *J. Virol. Methods* 179, 281–287.