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Rapid Approaches for Diagnosis of Canine Distemper Virus in Live and Dead Dogs in Egypt

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BACKGROUND: Canine Distemper Virus infection (CDV) is a highly contagious disease of high morbidity and mortality rates in dogs. The causative virus is CDV which is a Morbillivirus, CDV is a pantropic virus characterized by multisystemic infection and high case fatality, with worldwide distribution, so rapid diagnosis and quarantine of the infected dogs with starting suitable treatment was required. **The aim:** this study aimed to achieve rapid diagnosis of canine distemper virus infection on ante mortem and post mortem aspects. **Material and Methods:** One healthy control disabled dog, 53 infected dogs with suggestive clinical signs for CDV infection were checked by (a) clinical examination; (b) Rapid immunochromatgrophy (IC) onconjunctival swabs (c) qRT- PCR on blood and tracheal exudates to confirm presence or absence of CDV, by expression analysis of CDV-F gene. Then all 53 examined dogs isolated and received supportive treatment but all died and disabled controlled one exposed to soft death. qRT-PCR were conducted on tissue samples from all 54 dogs for detection CDV-F gene expression values in different tissues.(d) Statistical analysis to study effect of sex and breed using Chi-square test, to evaluate sensitivity, specificity, accuracy, PPV, negative PV, respectively of used test and prevalence of CDV. **Results:** Clinical signs suggestive for CDV infection recorded in all 53 examined dogs, 24 of 54 dogs were positive for IC. Gene expression analysis test detected high values for CDV- F gene expression in tracheal exudates and blood samples of 36 live dogs, while the expression values were also high in tissue samples from different organs of 36 dead dogs. Statistical comparison of IC to qRT-PCR showed that values were 72%, 100%, 81.4%, 100% and 64.2 for sensitivity, specificity, accuracy, PPV, negative PV, respectively. No effect of sex , age, and breed on results using Chi-square test. Prevalence of CDV infection was 66% among population of this study. **Conclusion:** this study concluded that detection of clinical signs suggestive for CDV with application of IC and qRT-PCR together should be applied as rapid diagnosis on ante mortem level, while qRT-PCR could be used for rapid post mortem diagnosis of CDV infection.

Keywords: Canine Distemper Virus, CDV, qRT-PCR, Immune-chromatography, Dogs, Egypt

Introduction

Canine Distemper viral (CDV) infection is a highly contagious febrile disease affect dogs. The disease presented as acute and subacute forms manifested clinically by signs of: respiratory manifestations, hyperkeratosis, neurological signs, systemic troubles or combination of them [1,2]. The disease characterized by high morbidity and mortality rates [1,3,4].

CD caused by CDV which is a Morbillivirus belongs to Morbillivirus Group, Family Paramyxovirridae, CDV is a negative single strand RNA. Enveloped, RNA of the virus encoding for 6 structural proteins: Fusion protein (F), Haemagglutinin protein (H), large polymerase (L), nucleoprotein capside (N), protein envelops matrix protein (M) and Phosphoprotein (P) [4,5].

Infection occurs via inhalation, CDV invade upper respiratory tract, lymphoid tissues (6), replicating inside macrophage and monocyte cells [7]. Incubation period varies from one to four weeks depend up on immune-status of dogs and strain virulence [6,8].

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Infected dogs develop a diphasic fever [6,8], in the first viraemic stage generalized infection of all lymphoid tissues characterized by transient fever with lymphoid depletion, lymphopenia, leucocyt ic necrosis and dysfunction end by immune-suppression [6,9,10]. In the second viraemic stage secondary viraemia associated with fever and mass infection of all parenchymal tissues, e.g. respiratory tract, GIT, CNS, skin. During the second stage various clinical signs may be founded as nasal discharge, anorexia, conjunctivitis, gastrointestinal tract signs, respiratory signs and neurological disturbance [6,11,12]. Interstitial pneumonia and rhinitis is sequel of respiratory tract infection, vomiting, diarrhea and dehydration caused by GIT tract infection, hyperkeratosis of skin.

Neurological signs in the form of hyperesthesia, cervical rigidity, seizures, paraparesis, sensory ataxia and cerebraller signs which developed as result of distribution of CDV in CNS tissue [13-15].

Diagnosis of CDV infection depend mainly up on isolation of the virus in tissue culture (canine cells) but this is a time consuming, taking several days to week [16)]. In spite of vaccination against canine distemper had applied for many decades, CDV infection still remain fatal disease of dogs [17].

Because of CDV causes a contagious disease of high mortality rates. The need for the use of rapid techniques is urgent, in order to isolation and quarantine of infected dogs with starting early treatment [18].

No published data regarding diagnosis of CDV in Egypt, in addition to importance of establishing definitive diagnostic system for the control of this disease is urgent. So this study aimed to set a definitive scheme for accurate, rapid diagnosis including clinical signs, the use of immune chromatography (IC) and quantitative Real Time-PCR (qRT-PCR) as rapid test for detection of CDV even in low log of viraemia in live and dead dogs.

Material and Methods

Duration of the study

This study has been conducted during the period between January 1, 2015 and October 30, 2018.

Ethical approval and informed consent

We informed and received the permission of the owners of dogs included in this study for taking samples used in this work. Samples were collected as per standard sample collection procedure without any harm to animals. We received agreement of owner of disabled control puppy for applying soft death, also we received agreement of all dog owners included in this study for using isolated diseased dogs for scarification,taking different tissue samples.

Chemicals

For molecular analysis, Trizol was bought from Invitrogen (Carlsbad, CA, USA). The reverse transcriptase and polymerase chain reaction (PCR) kits were obtained from Fermentas (Glen Burnie, MD, USA). For IC analysis, direct IC kits were purchased from Bionote Inc., Korea.

Examined animals

(a) 53 diseased dogs were examined. Each dog was examined clinically to detect the clinical manifestations of the disease. (b) One congenitally disabled healthy German Shepherd puppy of 90 day age old suffered from congenital disability in his hind limb and according to private personal request of the owner to make soft death of his disabled puppy [19, 20].

Clinical examination

Fifty three infected dogs and a healthy control disabled puppy were received at a Clinic at 6 October district located in Giza Governorate, Egypt. History of the examined dogs including breed, sex, age, past medical data history, and registered vaccination were recorded. Dogs subjected to general and specific clinical examination according to Gaskell et al. [1]. The severity of the clinical signs observed in this study was recorded as severe, moderate , mild or acute or sub-acute forms.

Sampling

Conjunctival swabs were collected from all clinically infected cases dogs and a healthy control disabled puppy was checked by rapid IC test (rapid CDV Ag test kit for qualitative detection of viral antigen in samples of all examined cases). For detection of CDV-F gene expression values on tracheal exudates and blood of all live dogs.

After death, liver, spleen, myocardium, lung, brain tissues and lymph nodes (mediastinal and retropharyngeal) were collected from all infected dogs and a healthy control disabled puppy after death. These dogs presented clinical signs of canine distemper virus infection. Collected samples were stored in 2 ml microtubes at a temperature of -80°C.

Immune-chromatography (IC)

Direct IC (the antigen rapid CDV Ag test kit, Bionote Inc., Korea) for the qualitative detection of Canine Distemper antigen in Canine conjunctival swabs were carried out on 53 infected dogs and a healthy control disabled puppy samples showing clinical signs of CDV viral infection [21].

Molecular study

Extraction of total RNA and cDNA synthesis

Tracheal exudates and blood from live dogs (n=54), in addition to liver, spleen, myocardium, lung, brain tissues and lymph nodes (mediastinal and retropharyngeal) samples were collected from all infected dogs after death and control disabled dog after soft death (n=54) according to Ramsay and Wetzel [19] and Reilly [20], and used for RNA extraction using TRIzol® Reagent (Invitrogen, Germany) Kit. The isolation method was carried out according to the manufacturer's instructions of the above Kit. Approximately 50 mg of the samples were homogenized in 1 ml of TRIzol® Reagent in Eppendorf tubes. Afterwards, total RNA was dissolved and preserved in diethylpyrocarbonate (DEPC)-treated water up to use [22, 23].

To assess the RNA yield and purity of the total RNA, RNAse-free DNAse I (Invitrogen, Germany) was used to digest DNA contamination. A small drop of isolated RNA was examined photospectrometrically at 260nm. The purity of total RNA was determined between 1.8 and 2.1 mg to be good purified when it examined by spectrophotometer at the 260/280 nm ratio. To avoid RNA damaging, aliquots of RNA were prepared after isolation for either reverse transcription reaction or otherwise for storing at -80°C up to use.

To synthesize the complementary DNA (cDNA) isolated RNA from all samples were reverse transcribed into cDNA. The reaction volume was carried out in 20 µl. The reaction volume was prepared according to the instructions of the RevertAidTM First Strand cDNA Synthesis Kit (MBI Fermentas, Germany). The reverse transcription (RT) reaction was performed for 10 min at 25°C. Afterwards, the tubes of the reaction were put in thermo-cycler machine for 60 min at 42°C, and then the reaction was terminated for 5 min at 99°C. The PCR products containing the cDNA were kept at –80°C up to use for DNA amplification [22, 23].

Quantitative Real Time-PCR (qRT-PCR)

Primer design

Specific primers used in this study were designed for different regions of F gene which codes virus fusion protein using primer3 program as illustrated in Table 1.

A StepOne Real-Time PCR System (Applied Biosystem, USA) was used to assess the copy of the cDNA of tracheal exudates, liver, spleen, myocardium, lung, brain tissues and lymph nodes (mediastinal and retropharyngeal) to detect the expression values of the Canine Distemper virus F-gene. A volume of 25 µL of reaction mixtures was prepared containing 12.5 µl of SYBR® green (TaKaRa, Biotech. Co. Ltd.), 0.5 µl of forward and reverse primers, 6.5 µL DNA-RNA free water, and 2.5 µl of the synthesized cDNA. A melting curve of the reaction was performed for each qRT-PCR termination at 95.0°C to assess the quality of the primers. To verify that the reaction of the qRT-PCR does not have any contamination PCR tubes containing non template control were used. The sequences of specific primers of the Canine Distemper virus F-gene were used Table 1. The relative quantification of the target gene to the reference (GAPDH, [24] was determined by using the 2−ΔΔCT method [22, 23].

TABLE 1. CDV primers used in these study.

.No	Gene	Primer Name	Oligonucleotide sequence (′3-′5)	Estimated (size[a] (bp product	Reference/ Accession number
1	CDV-F	F	CGTCGCAAATTGTGCTTCTA	1579 - 1821	M21849.1
		R	AGGGCGTTCCCTAAGTTTGT		
2	GAPDH	F	GAGAAAGCTGCCAAATATG	193	Tarlinton [24]
		R	CCAGGAAATGACCTTGACA		

[a]Based on available CDV genome sequences. CDV=Canine Distemper virus

Statistical Analysis

The statistical analysis of the obtained results was done using Chi-square test to study the effect of age, sex and breeds on results of different tests. Sensitivity, specificity, accuracy, P ositive predictive value (PPV) and negative predictive values (NPV) of used diagnostic test and prevalence of the disease in population of this study were calculated according to Smith [25].

Results

Clinical examination

The severity of the disease varied from sever acute to subacute moderate form or latent mild form that was fatal usually in puppies.

Fifty three naturally infected puppies, 1 disabled healthy control male German Shepherd of age 2 to 4 months of history with no vaccination against Distemper virus were kept under close observation and taking all sanitary and epidemiological measures for prevention of infection spread according to recommended protocol for Distemper virus control.

The observed clinical signs were:

- *Non neurological signs:* fever 40 – 41.7 ºC serous – mucopuralant nasal and conjunctival discharge, coughing, dyspnea, vomiting and diarrhea as shown in Table 2 and Fig. 1.

TABLE 2. The clinical signs detected in examined dogs.

Breed	No. of cases	Breed size		Age (mon)	Sex		Fever (ºC)	Clinical Signs	
		Small	Large		Male	Female		Non neurological*	Neurological**
German Shepherd	16	-	16	2-3	9	7	40-41.6	Severe	Moderate
Golden Retriever	15	-	15	2-3	7	8	40-41.5	Severe	Severe
Yorkshire	3	3	-	2-3	2	1	40-41	Moderate	Severe
Labrador Retriever	12	-	12	2-3	6	6	40-41.3	Severe	Moderate
Griffon	5	5	-	2-3	2	3	40-41.5	Severe	Severe
Boxer	3	-	3	3-4	2	1	40-41.7	Mild	Moderate
Total	54	8	46		28	26			

* Fever 40 – 41.7 ºC serous – mucopuralant nasal and conjunctival discharge, coughing, dyspnea, vomiting and diarrhea
** Shewing gums, seizures, circling, continues fits of high voices, later end by limb ataxia and weakness, head tremors, head tilt, depression. The severity of clinical signs evaluated as mild, moderate and severe.

Fig. 1: German Shepherd puppy infected with CDV with chronic respiratory form (non-neurological form)

- *Neurological signs:* includes shewing gums, seizures, circling, continues fits of high voices, later end by limb ataxia and weakness, head tremors, head tilt, depression, behavior changes end by death as shown in Table 2.

Immune-chromatography (IC)

All hospitalized infected puppies that showed clinical signs were checked by rapid Immune-chromatography (IC) for qualitative detection of canine distemper viral antigen in conjunctival discharge, the 26 mentioned puppies were positive when checked by rapid IC test as mentioned in Fig. 2 and Table 3, while control disabled puppy were negative.

Molecular study

Expression levels of CDV-F gene in dog tissues

Fig. 2. Immune chromatography test kit showing positive results for CDV infection

TABLE 3. Positive and negative male and females dogs examined for CDV using Immune-chromatography.

Case	Percentage (%)	
	Male	Female
Positive	14 (50)	12 (46)
Negative	14 (50)	14 (54)
Total	(100) 28	26 (100)

Figure 3 represents the levels of Distemper virus F-gene expression in different Tracheal exudates and blood samples of live dogs (Fig. 3). The CVD-F gene expression values were higher significantly in tracheal exudates than in blood samples with ($P<0.05$) while expression values undetectable in control dog samples.

Figure 4 represents the levels of Distemper virus F-gene expression in liver, spleen, myocardium, lung, brain and retropharngial & mediastinal lymph nodes samples of infected dogs.

The results revealed that the Distemper virus F-gene was of low values and undetectable in all control dog tissues. However, expression values of Distemper virus F-gene in different infected dog tissues were significantly higher when compared with the control dog tissue samples. In addition to the expression values of Distemper virus F-gene increased significantly with differences of $P<0.05$ in lung tissues& lymph nodes then brain compared with liver, myocardium and spleen (Fig. 4).

Statistical analysis

Results of statistical analysis proved that sex, age and breed have no significant effect on results of both IC and qRT-PCR. qRT-PCR is considered as gold standard of this study. Table (4) represents the comparison between IC and qRT-PCR showing 72%, 100%, 81.4.65%, 100% and 64.2% on sensitivity, specificity, accuracy, positive predictive value and negative protective value. Prevalence of CDV infection among population of this study was 66%

Discussion

Clinical examination considered as the first utility for diagnosing of CDV infection in this study , it detected clinical signs in the form of non neurological and neurological signs which were suggestive for CDV infection as stated by Gaskell et al.[1].But these recorded clinical signs clinically confused with other neurological and non neurological respiratory diseases as confirmed by Shaw and Ihle [26].

So, IC applied as a rapid field test reliable for diagnosis of CDV infection in veterinary clinics

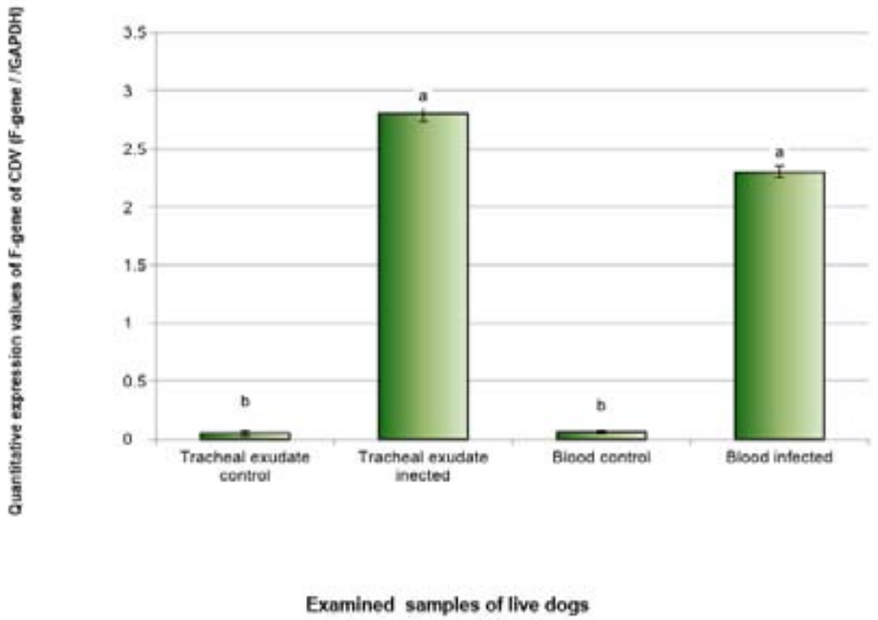

Fig. 3. Expression levels of F-gene of CDV in different live dog samples. Data are presented as mean ± SEM. a,b followed by different superscripts are significantly different (P≤0.05).

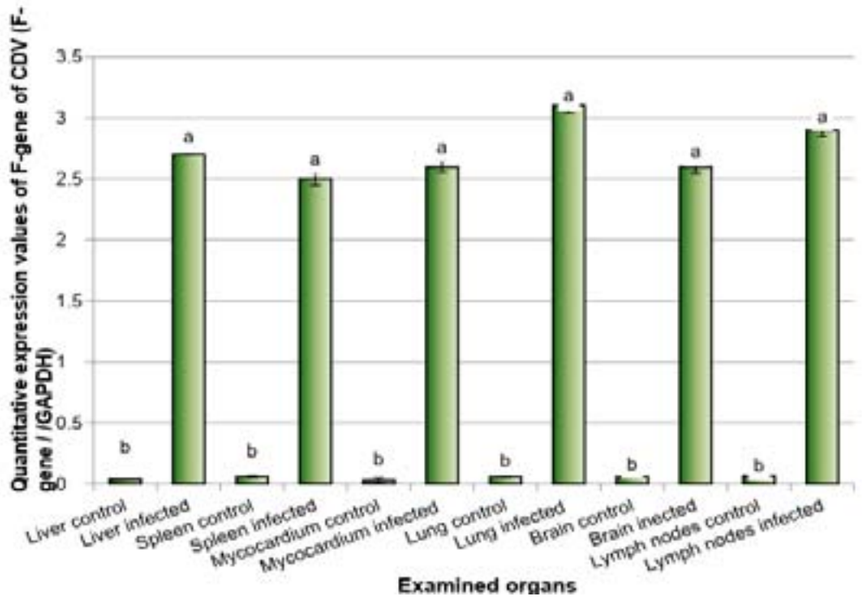

Fig. 4. Expression levels of F-gene of CDV in different dead dog samples. Data are presented as mean ± SEM. a,b followed by different superscripts are significantly different (P≤0.05).

TABLE 4. Comparison between immune-chromatography (IC) according qRT-PCR findings of examined cases of CDV in live dogs.

Method of identification		IC		Total
		Positive	Negative	
qRT-PCR	Positive	26(T+)	10(F-)	36
	negative	0(F+)	18(T-)	18
	Total	26	28	54

(T+): True positive, (T-): True negative, (F+): False positive, (F-): False negative
Sensitivity= 72% Positive pre-detective values (+PPV)= 100%
Specificity= 100% Negative pre-detective values (-NPV= 64.2%
Accuracy= 81.4%

& hospitals. It proved to be specific, easily in performance for detection of CDV antigen in conjunctival swabs of examined dogs as reported by An et al. [21].

IC succeeded in detection of 26 infected cases among 54 examined dogs, due to lack in its sensitivity for detection the infected cases harboring low concentration of viremia below 105.5 $TCID_{50}$/ml [27].

In this work Gene expression analysis test (qRT-PCR) tried as highly sensitive, specific test with high accuracy in detection of CDV-F gene expression using specific primer on blood and tracheal exudate of examined dogs as recommended by . Elia et al. [18] and Budaszewski & von Messling [28].

qRT-PCR in this study succeeded in detection of 36 infected cases among 54 suspected cases due to its high sensitivity in detection of CDV- F gene expression even in very low virus concentration which may reach to 10^2 as confirmed by Elia et al. [18].

These results proved that gene expression analysis test was an accurate, conclusive, confirmatory, qualitative test in diagnosis of CDV infection in live dogs as confirmed by many authors [Elia et al. [18] and Shaw & Ihle [26]. Moreover that qRT-PCR was a sensitive anti mortem diagnostic technique especially reliable in sub acute and chronic stages of CDV infection [17, 18, 28-30].

Results of qRT-PCR when applied in liver, spleen, myocardium , lung , retropharngeal & mediastinal lymph nodes and brain tissue of dead dogs, showed that CDV-F gene expression values were high in lung & lymph nodes than that values of liver, spleen, myocardium and brain significantly (P<0.05), while there is no significant difference between expression values of liver, spleen, myocardium, and brain tissues agreed with Elia et al. [18].

The above mentioned results recommend the use of qRT-PCR as quantitative assay for measuring CDV load & distribution in different infected organs by estimating CDV-F gene expression quantitatively, with high accuracy because it can detect CDV even in level 10^2 to 10^8 . So, qRT-PCR served as reproducible assay for studying pathogenetic mechanisms induced by CDV [17, 18, 30]. Moreover, the gene expression values in blood and tracheal exudate were high as that recorded in lung tissue, these findings nominate qRT-PCR as rapid molecular assay for studying CDV spread and shedding [18, 28, 30-35].

Conclusion

This study concluded that detection of clinical signs suggestive for CDV, application of rapid IC and molecular assaying by qRT-PCR could serve as rapid, accurate, confirmatory methods of CDV diagnosis on ante-mortem level without need for CDV isolation, while qRT-PCR was considered as accurate assay for rapid diagnosis on post-mortem level.

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Conflict of Interest Statement

The author whose name is listed immediately below certify that he has no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Author contribution

The author performed the study plan and design. Romane Adieb Awad collected the samples from the clinic, and had carried out the clinical examination and IC laboratory work. Romane Adieb Awad carried out the work of clinical evaluation of the diseased dogs, treatment, clinical follow-up and assessment. He also has carried out the molecular work included in this study. Romane Adieb Awad had done all issues of writing, revising, and improvement of the article for publication.

Ethical consideration

The owners of dogs were informed and permission was received from them. The dogs included in this study for taking samples used in this work. Samples were collected as per standard sample collection procedure without any harm to animals. Individual permission received from the owner of the disabled control puppy and up on his request to do soft death of his disabled control puppy. The proposal of this study had approval from National Research Center committee no: 10/01/2015.

References

1. Gaskell, R.M., Tennant, B., Bennett, M. and Willoughby, K. Feline and Canine Infectious Diseases. Published by Iowa State Press, Ames, IA., 1996.

2. Appel, M.J.G. and Summers, B.A., Pathogenicity of morbilliviruses for terrestrial carnivores. *Vet. Microbiol*., **44**, 187-191 (1995).

3. FroÈlicha, K., Czupalla, O., Haas, L., Hentschke, J., Dedek, J. and Fickel, J.E., Epizootiological investigations of canine distemper virus in free-ranging carnivores from Germany. *Veterinary Microbiology*, **74**, 283 – 292 (2000).

4. Greene, C.E. and Appel, M.J.G., Canine distemper: In: Greene, C.E. (Ed.) Infections Diseases of the dog and cat. W.B. Saunders, Philadelphia, pp. 25-41. (2006).

5. Pardo, I.D.R., Johnson, G.C. and Kleiboeker, S.B., Phylogenetic Characterization of Canine Distemper Viruses Detected in Naturally Infected Dogs in North America. *Journal of Clinical Microbiology*, 5009–5017 (2005).

6. Krakowka, S., Higgins, R.J. and Koestner, A., Canine distemper virus: review of structural and functional modulations in lymphoid tissues, *Am. J. Vet. Res.,* **41**, 284–292 (1980).

7. Appel, MJG, Pathogenesis of canine distemper. *Am. J. Vet. Res.,* **30**, 1167-1182 (1969).

8. Wright, N.G., Cornwell, H.J., Thompson, H. and Lauder, I.M., Canine distemper: current concepts in laboratory and clinical diagnosis. *Vet. Rec.,* **94**, 86–92 (1974).

9. Schobesberger, M., Summerfield, A., Doherr, M.G., Zurbriggen, A. and Griot, C., Canine distemper virus-induced depletion of uninfected lymphocytes is associated with apoptosis, *Vet. Immunol. Immunopathol*., **104**, 33–44 (2005).

10. Qeska, V., Barthel, Y., Herder, V., Stein, V.M., Tipold, A., Urhausen, C., Günzel-Apel, A.R., Rohn, K., Baumgärtner, W., Beineke, A., Canine distemper virus infection leads to an inhibitory phenotype ofmonocyte-derived dendritic cells in vitrowith reduced expression of co-stimulatory molecules and increased interleukin-10 transcription. *PLoS One* **9**, e96121 (2014).

11. Appel, M.J., Distemper pathogenesis in dogs. *J. Am. Vet. Med. Assoc.,* **156**, 1681–1684 (1997).

12. Decaro, N., Camero, M., Greco, G., Zizzo, N., Tinelli, A., Campolo, M., Pratelli, A. and Buonavoglia, C., Canine distemper and related diseases. Report of a severe outbreak in a kennel, *New Microbiol*., **27**, 177–181 (2004).

13. Deem, S.L., Spelman, L.H., Yates, R.A. and Montali, R.J., Canine distemper in terrestrial carnivores: a review, *J. Zoo Wildl. Med.,* **31**, 441–451 (2000).

14. Baumgärtner, W., Boyce, R.W., Alldinger, S., Axthelm, M.K., Weisbrode, S.E., Krakowka, S. and Gaedke, K., Metaphyseal bone lesions in young dogs with systemic canine distemper virus infection. *Vet. Microbiol.,* **44**, 201–209 (1995).

15. Von Rüden, E.L., Avemary, J., Zellinger, C., Algermissen, D., Bock, P., Beineke, A., Baumgärtner, W., Stein, V.M., Tipold, A. and Potschka, H., Distemper virus encephalitis exerts detrimental effects on hippocampal neurogenesis, Neuropathol. *Appl. Neurobiol.,* **38**, 426–442 (2012).

16. Kim, Y.H., Cho, K.W., Youn, H.Y., Yoo, H.S. and Han H.R., Detection of canine distemper virus (CDV) through one step RT-PCR combined with nested PCR. *J. Vet. Sci.,* **2**, 59–63 (2001).

17. Jóźwik, A. and Fryms, T., Comparison of the immunofluorescence assay with RT-PCR and nested PCR in the diagnosis of Canine Distemper. *Veterinary Research Communications*, **29**, 347-359 (2005)

18. Elia, G., Decaro, N., Martella, V., Cirone, F., Lucente, M.S., Lorusso, E., Trani, L.D. and Buonavoglia, C., Detection of canine distemper virus in dogs by real-time PCR. *Journal of Virological Methods,* **136**, 171-176 (2006).

19. Ramsay, E.C. and Wetzel, R.W., Comparison of five regimens for oral administration of medication to induce sedation in dogs prior to euthanasia. *J Am Vet Med Assoc,* **213** (2), 240–242 (1998).

20. Reilly, J.S., Euthanasia of animals used for scientific purposes. Australian and New Zealand Council for the Care of Animals in Research and Teaching, Adelaide, Australi, (1993).

21. An, D.J., Kim, T.Y., Song, D.S., Kang, B.K., Park, B.K. An immunochromatography assay for rapid antemortem diagnosis of dog suspected to have canine distemper, *J. Virol. Methods,* **147** (2), 244-249 (2008).

22. Awad, R.A., Khalil, W.K.B. and Attallah, A.G., Epidemiology and diagnosis of feline panleukopenia virus in Egypt: Clinical and molecular diagnosis in cats. *Veterinary World*, **11** (5), 578-584 (2018).

23. Awad, R.A., Khalil, W.K.B. and Attallah, A.G., Feline panleukopenia viral infection in cats: Application of some molecular methods used for its diagnosis. *Journal of Genetic Engineering and Biotechnology,* **16**, 491–497 (2018).

24. Tarlinton, R.E., Barfoot H.K., Allen C.E., Brown K., Gifford R.J. and Emes R.D., Characterisation of a group of endogenous gammaretroviruses in the canine genome. *Veterinary Journal*, **196,** 28–33 (2013).

25. Smith, R.D., Veterinary Clinical Epidemiology. 3rd ed. CRC Press, Boca Raton., (2005).

26. Shaw, D.H. and Ihle S.L., Small animal internal medicine, published by Williams and Wilkins, chapter 47, p 464-468 (1997).

27. Soma, T., Soma, T., Ishii, H., Hara, M., Ohe, K, Hagimori, I, Ishikawa, Y, Taneno, A. Detection of Canine Distemper virus antigen in Canine serum and its application to diagnosis. *Vet. Records*, **18**, 499-501 (2003).

28. Budaszewski, R.D.F. and Von Messling, V. Morbillivirus Experimental Animal Models: Measles Virus Pathogenesis Insights from Canine Distemper Virus. *Viruses.,* **8** (10), 274 (2016).

29. Wang, J., Wang, J., Li, R., Shi, R., Liu, L. and Yuan, W. Evaluation of an incubation instrument-free reverse transcription recombinase polymerase amplification assay for rapid and point-of-need detection of canine distemper virus. *J. Virol. Methods*., **260**, 56-61 (2018).

30. Wang, J., Wang, J., Li, R., Liu, L. and Yuan, W. Rapid and sensitive detection of canine distemper virus by real-time reverse transcription recombinase polymerase amplification. *BMC Vet Res.,* **13** (1), 241 (2017).

31. Nemeth, N.M., Oesterle, P.T., Campbell, G.D., Ojkic, D. and Jardine, C.M., Comparison of reverse-transcription real-time PCR and immunohistochemistry for the detection of canine distemper virus infection in raccoons in Ontario, Canada. *J. Vet. Diagn. Invest.*. **30** (2), 319-323 (2018).

32. Sehata, G., Sato, H., Ito, H., Imaizumi, Y., Noro, T. and OISHI E., Use of quantitative real-time RT-PCR to investigate the correlation between viremia and viral shedding of canine distemper virus, and infection outcomes in experimentally infected dogs. *J. Vet. Med. Sci.,* **77** (7), 851–855 (2015).

33. Willi, B., Spiri, A.M., Meli, M.L., Grimm, F., Beatrice, L., Riond, B., Bley, T., Jordi, R., Dennler, M. and Hofmann-Lehmann, R., Clinical and molecular investigation of a canine distemper outbreak and vector-borne infections in a group of rescue dogs imported from Hungary to Switzerland. *BMC Vet. Res.,* **11**, 154 (2015).

34. Riley, M.C. and Wilkes, R.P., Sequencing of emerging canine distemper virus strain reveals new distinct genetic lineage in the United States associated with disease in wildlife and domestic canine populations. *Virol. J.,* **12**, 219 (2015).

35. Wang, J., Li, R., Shi, R., Liu, L. and Yuan, W., Evaluation of an incubation instrument-free reverse transcription recombinase polymerase amplification assay for rapid and point-of-need detection of canine distemper virus. *J. Virol. Methods*., **260**, 56-61 (2018).

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تقنيات سريعة لتشخيص فيروس الدستمبر فى الكلاب الحية والميتة فى مصر

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تعتبر عدوى الاصابة بفيروس الدستمبر فى الكلاب من الامراض الوبائية ذات معدل الاصابة والنفوق العالى فى الكلاب. مسبب المرض هو فيروس الدستمبر وهو عبارة عن فيروس موربيلى وهو فيروس يصيب اجهزة مختلفة فى جسم الكلاب ويؤدى الى معدل وفاة عالى وهو منتشر فى اماكن كثيرة على مستوى العالم. لذلك فان التشخيص السريع وعزل الكلاب المريضة والابتداء بعلاجها ضرروة ملحة. لذلك هذه الدراسة تهدف الى انجاز تشخيص سريع لفيروس الدستمبر فى الكلاب على مستوى الكلاب الحية ومستو الكلاب الميتة. هذهالدراسة شملت كلب معاق سليم صحيا و٥٣ كلب مصاب باعراض اشتباه بالاصابة بفيروس الدستمبر. هذه الكلاب تم فحصهم بالفحص الاكلينيكى واختبار الامينو كروماتوجرافى السريع على مسحات من العين واختبار تفاعل البلمرة المتسلسل العكسى الكمى فى ذات الوقت على عينات دم وافرازات القصبة الهوائية من هذه الكلاب للتاكد من وجود او عدم وجود فيروس الدستمبر. وبعد ذلك كل اعداد ال ٥٣ كلب المصاب تم عزلهم واعطائهم العلاج المناسب ولكن كلهم ماتوا. والكلب المعاق السليم تم انهاء حياته بالموت الرحيم. اختبار تفاعل البلمرة المتسلسل العكسى الكمى فى ذات الوقت تم عمله على عينات من انسجة ال ٥٤ كلب للتاكد من وجود جين أف الخاص بفيروس الدستمبر فى الانسجة المختلفة. تم عمل التحليل لدراسة تاثير الجنس والسلالالة باستخدام مربع كاى تربيع وتم حساب الحساسية والخصوصية والدقة للاختبارات المستخدمة على التوالى. كذلك تم حساب معدل تواجد فيروس الدستمبر. اظهرت نتائج الفحص الاكلينيكى وجود اعراض عدوى الاصابة بفيروس الدستمبر فى ٥٣ كلب واظهر اختبار الامينو كروماتوجرافى ان ٢٤ كلب من جملة ٥٤ كلب مصابين فى حين اظهر اختبار تفاعل البلمرة المتسلسل العكسى الكمى فى ذات الوقت قيم عالية لوجود الجين أف فى افرازات القصبة الهوائية والدم المجمعين من ٣٦ كلب احياء بينما اظهر اختبار تفاعل البلمرة المتسلسل العكسى الكمى فى ذات الوقت وجود قيم عالية لتكرارات الجين أف فى عينات الانسجة المختلفة والمجمعة من ٣٦ كلبا ميتا. اظهر المقارنة الاحصائية التى تمت بين اختبار الامينو كروماتوجرافى و اختبار تفاعل البلمرة المتسلسل العكسى الكمى فى ذات الوقت ان قيمة الحساسية والخصوصية والدقة كانت ٧٢٪ و ١٠٠٪ و &٨١ لاختبار الامينو كروماتوجرافى. اوضحت النتائج الاحصائية انه لا يوجد تأثير للجنس او العمر او السلالة على النتائج. معدل تواجد فيروس الدستمبر كان ٦٦٪ بين التعداد الذى شملته الدراسة. الخلاصة: توصلت هذه الدراسة الى ان وجود الاعراض الاكلينيكية التى تجسد صورة الاصابة بفيروس الدستمبر مع تطبيق اختبار اختبار الامينو كروماتوجرافى و اختبار تفاعل البلمرة المتسلسل العكسى الكمى فى ذات الوقت معا يجب استخدامهم للتشخيص السريع والدقيق فى الكلاب الحية فى حين ان اختبار تفاعل البلمرة المتسلسل العكسى الكمى فى ذات الوقت يجب ان يستخدم للتشخيص السريع لعدوى فيروس الدستمبر فى الكلاب الميتة.