THE IRAQI JOURNAL OF
VETERINARY MEDICINE
eISSN:2410-7409
pISSN: 1609-5693

OPEN ACCESS

Molecular Detection of Canine Distemper Virus in Dogs in Baghdad Province, Iraq

Khalefa A Mansour*[1], Saleem A Hasso[2]

[1]Department of Veterinary Internal and Preventive Medicine, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Qadisiyah, Iraq, [2]Department of Veterinary Internal and Preventive Medicine, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

***Correspondence**:
khalefa.mansour@qu.edu.iq
saleemaminhasso@yahoo.com

Received: 23 August 2021
Accepted: 27 December 2021
Published: 28 December 2021

Cite
Mansour KA, Hasso SA. Molecular detection of canine distemper virus in dogs in Baghdad city, Iraq. Iraqi J. Vet. Med. 2021; 45(2): 46-50.

A B S T R A C T

Canine distemper (CD) is an infectious disease that affects dogs and is extremely contagious and lethal, with a high mortality and morbidity rates. It infects a broad variety of animals, including primates, cetaceans, and carnivores causing a multi-systemic pathological condition. This study aimed to detect canine distemper virus (CDV) in blood samples of dogs clinically suspected with distemper at the Baghdad Veterinary Hospital, Baghdad, Iraq. CDV nucleoprotein gene (N) was detected in the whole blood of 46 dogs using reverse transcription-PCR (RT-PCR). The partially amplified (591 bp) fragment of the N gene was detected in 12 of 46 (26%) blood samples of dogs examined. Based on the partial sequencing data of the N gene, three local isolates might be similar to the NCBI-BLAST reference CDV virus isolates FJ977579.1 China, AF378705.1 USA, and AF305419.1 UK, while other strains EU072200.1 Hungary, AF164967.1 Switzerland, KU578257.1 Germany, and AB474397.1 Japan were found to be rather distinct. The isolates displayed a higher level of similarity with the Snyder Hill CDV strain and Onderstepoort CDV strain. There was less homology with the CDV strain A75/17 of Switzerland and 007Lm CDV strain of Japan. In conclusion, this study confirmed that CDV infection is present in domestic dogs in Iraq. This may indicate a risk of the disease spreading to parts of the country that may be disease-free.

Keywords: CDV, detection, RT-PCR, phylogenetic, nucleocapsid gene

INTRODUCTION

Canine distemper (CD) is an infectious respiratory illness that affects young dogs, particularly those between the ages of three and six months, resulting in high morbidity and, in those that have not been vaccinated, high mortality. The infection is mostly transmitted during the winter. Since 1760, it has been considered to be an acute or subacute infectious febrile disease defined by clinical signs of respiratory, gastrointestinal, integumentary, and central nervous systems, or a combination of these symptoms in domestic and wild dogs (1, 2).

Canine distemper virus (CDV) is the causative agent of CD. It is an enveloped viral particle with a diameter of 150 to 300 nm with a central core containing helices 15 to 17 nm in diameter (1). The CDV genome is a nonsegmented single-stranded negative sense RNA that encodes six structural proteins: nucleocapsid protein, phosphoprotein,

fusion protein, hemagglutinin protein, matrix protein, and large polymerase protein. CDV belongs to the family *Paramyxoviridae* and the genus *Morbillivirus* (3, 6, 7). The CDV mainly replicates in respiratory tract lymphatic tissues. Lymphopenia and a short-term fever emerge 3 to 6 days after infection (3–5).

In unvaccinated puppies, diagnosis of this disease, whether subacute or acute, must be made dependent on clinical symptoms and history. However, it was difficult to tell the difference between this disease and other types of illnesses, such as kennel cough when the disease is in its early stages. Anti-CDV IgM antibody identification can be used to make a serologic diagnosis (8, 9). However, owing to a detectable IgM antibody titer to CDV after 3 weeks of vaccination, it remains a problem in vaccinated pets (9).

Currently, dogs exhibiting clinical symptoms of a chronically antibiotic-resistant respiratory disease, or a neurological disease are tentatively diagnosed with CD. In advance stages of infection, i.e., when the central nervous system has already been infected with CDV, convulsions, lack of coordination, and myoclonus develop as more specific symptoms for CD diagnosis. Furthermore, CD is a complex disorder to be identified due to its wide range of clinical manifestations. Therefore, early detection of infection is essential for isolating sick dogs and administering adequate care. For this reason, to identify a lesser amount of virus in early infection, a sensitive, rapid, and specific method is required. Different methods for detecting CDV antigen or RNA have been used, but the majority of them are time-consuming and complicated, and they may generate inaccurate results, making them inappropriate for antemortem diagnosis. New diagnostic methods are being developed as a result of recent advances in molecular biotechnological applications. The CDV RNA is detected in blood samples and cerebrospinal fluid of dogs suspected of developing the disease using a reverse transcription polymerase chain reaction (RT-PCR) (10, 11).

The aim of this study was to detect canine distemper virus for the first time in Iraq from blood samples of clinically ill dogs using a set of extremely specialized primer sequences.

MATERIALS AND METHODS

Sample Collection

Blood samples were collected from forty-six domestic dogs (most of them were not vaccinated) of both sexes at different ages which brought to Baghdad Veterinary Hospital between November 2020 and May 2021. Clinically, they were suspected of having CDV infection symptoms such as nasal discharge, excessive salivation, bronchitis, conjunctivitis, gastroenteritis, catarrhal pneumonia, and neurological disturbances.

The EDTA-treated blood samples were drawn into tubes, and 200 µL of the samples were used for viral RNA extraction. Before being used, the samples were stored at -70°C.

Viral RNA Extraction

The 46 blood samples were used to obtain the viral RNA using the AccuZol™ Total RNA extraction kit (Bioneer, Korea) according to the manufacturer's instructions. A 200 µL of the blood sample was loaded into a 1.5 mL Eppendorf tube, and 1 mL of AccuZol™ reagent was added. Then, 200 µL of chloroform was added and shacked. The supernatant was moved to a new Eppendorf tube, which was then filled with 500 µL of isopropanol. The tube was inverted 4-5 times to blend the mixture, and then incubated at 4°C for 10 min. The RNA pellet was allowed to dry after the aqueous phase was discarded for 5 min drying. After that, the RNA was treated with Diethyl pyrocarbonate (DEPC).

The RNA concentration and purity were measured using a NanoDrop™1000 spectrophotometer (Thermo Scientific, USA) at absorbance of 260/280 nm at ratio 1.8, which indicates pure RNA.

Primers

For identification of the N gene of the genus *Morbillivirus*, the primers were designed using NCBI GenBank primer design tool for the gene accession number EF375619.1. The predicted RT-PCR products were 591 bp. The primers were produced by Macrogen Company, Korea, and their sequences were as follows: Forward: 5'-CAA GAG GAC TCG GGA CCA AC-3', Reverse: 5'-TCT CCG ACC ACA CGT CTT TG-3'.

RT-PCR Conditions

OptiScript™ RT System RT/PCR PreMix (iNtRON Biotechnology, Korea) was used to conduct one step reverse transcription PCR. Overall, a volume of 20 µL, including 8 µL of one step reverse transcription-PCR PreMix, 1 µL of the RNA template and gene specific primers, and distilled water up to a total volume of 20 µL were added and mixed thoroughly inside an Eppendorf tube. PCR thermocycler conditions included: the reaction of reverse transcription for one cycle at a temperature of 37°C for 30 min, followed by pre-denaturation at 95°C for 5 min for one cycle, denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec, extension at 72°C for 30 min for 36 cycles, and final extension at 72°C for 5 min for one cycle. The product of RT-PCR was visualized under UV light after staining the1% agarose gel with ethidium bromide.

DNA Sequencing and Phylogenetic Analysis

The PCR products, three positive samples, were sequenced using the AB-DNA sequencing system, Korea (Bioneer Company). The MEGA6 software package was used to perform phylogenetic analysis (neighbor-joining) with bootstrap statistical support. The analysis was carried

out using the UpGMA tree (Arithmetic Mean) in Version MEGA 6.0 and new optional Version 1.20.0 of the NCBI Multiple Sequence Alignment Viewer.

RESULTS

RT-PCR

Partial amplification of the viral N gene using common primers in the one-step reverse transcription polymerase chain reaction revealed presence of 12 viruses out of 46 (26.0%) that belong to the genus *Morbillivirus* in the dogs' blood samples. Figure 1 shows bands of approximately 591bpas expected).

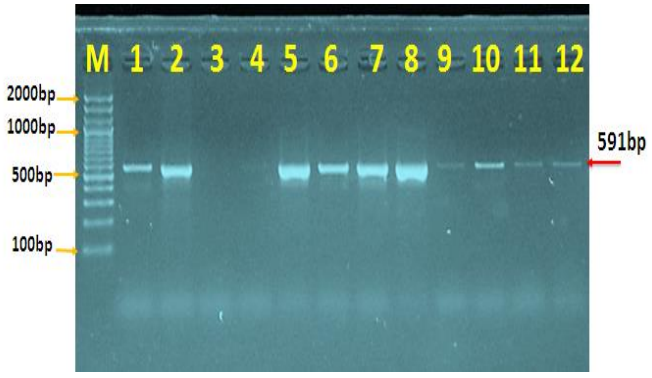

Figure 1. Agarose gel electrophoresis (stained with ethidium bromide) showing the RT-PCR products (591 bp) of the partially amplified N gene of *Morbillivirus* isolation. M, 100 bp DNA marker ladder; lanes 1-12, some positive samples

Blood samples were the ideal samples for CDV ante-mortem diagnosis. However, based on the common primers used, this study identified presence of viruses belong to the genus *Morbillivirus.* The results also showed that the dogs were infected even though they had received the vaccine against the canine distemper disease.

Sequencing and Phylogenetic Tree Analysis

The N gene partial sequences of the three isolates identified in this study were found to be identical to CDV. The accession numbers of these sequences were deposited in the GenBank databases under the accession numbers MW682324.1, MW682325.1, and MW682326.1.

Results of the phylogenetic tree constructed based on these partial N gene sequences of the local Iraqi isolates were found to be closely related to NCBI-BLAST reference CDV isolates FJ977579.1 China, AF378705 USA and AF305419.1 UK. While the other strains EU072200.1 Hungary, AF164967.1 Switzerland, KU578257.1 Germany, and AB474397.1 Japan were found to be distinct (Figure 2).

According to the NCBI Multiple Sequence Alignment (Figure 2), the local isolates displayed a higher level of similarity with the "Snyder Hill" CDV strain (97.83%) and "Onderstepoort" CDV strain (97.25%), there is less homology to CDV strain "A75/17" Switzerland and "007Lm" Japan CDV strain (96.25 %) (Table 1).

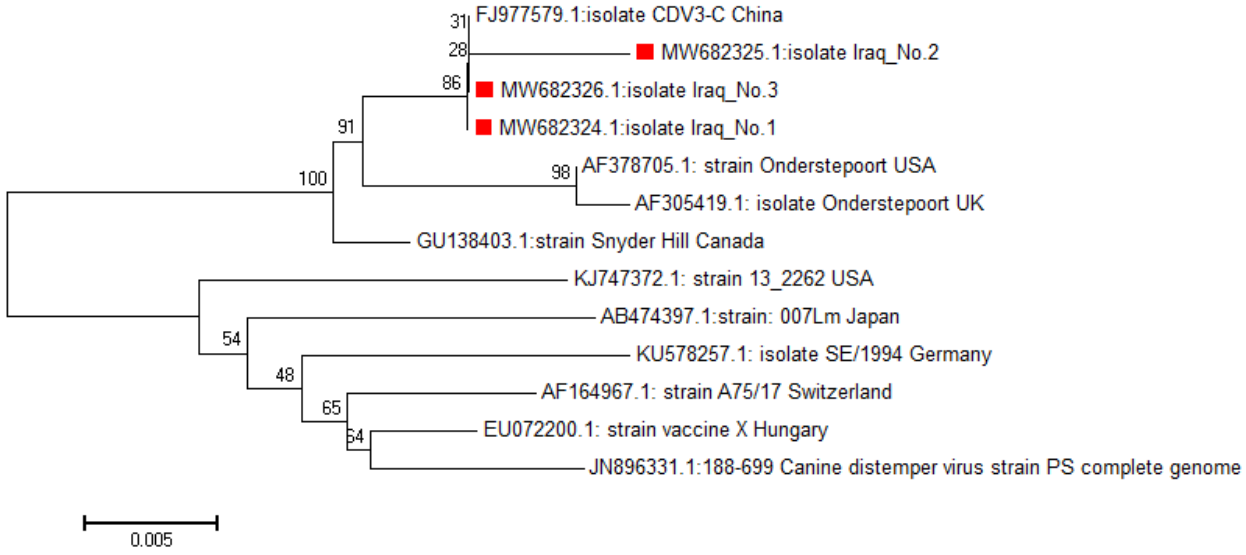

Figure 2. Phylogenetic relationship of an Iraqi local isolate, according to global reference strains. The results of phylogeny tree construction of local Iraqi CDV virus isolates revealed to be genetically similar to the NCBI-Blast reference CDV virus isolates (FJ977579.1 China, AF378705 USA, and AF305419.1 UK.), while others (EU072200.1 Hungary, AF164967.1 Switzerland, KU578257.1 Germany, and AB474397.1 Japan) were found to be distinct

Table 1. Sequence identity between the Iraqi strains compared with other global isolates on NCBI

Name	Sequence ID	Country	Source	Identity %	Coverage	Mismatch
Canine morbillivirus isolate Iraq_No.2	MW682325.1	Iraq	blood	97.65	100.00	12
Canine distemper virus isolate CDV3-C	FJ977579.1	China		97.84	100.00	11
Canine morbillivirus isolate Iraq No. l	MW682324.1	Iraq	Blood	97.83	99.41	11
Canine morbillivirus isolate Iraq No. 3	MW682326.1	Iraq	Blood	97.83	99.22	11
Canine distemper virus strain Snyder Hill	JN896987.1	USA	CDV_{SH}	97.84	100.00	11
Canine distemper virus strain recombina	GU138403.1	Ohio	cerebros	97.84	100.00	11
Canine distemper virus strain Orvderstep	AF378705.1	USA	CDV_{5804}	97.25	100.00	14
Canine distemper virus, isolate Orsderste	AF305419.1		Onderstepoort	97.25	100.0014	14
Canine distemper virus strain vaccine	EU072200.1	Hungary	(OND_{LP})	97.06	100.00	15
Canine distemper virus strain A75/17	AF164967.1	Switzerland	"A75/1"	96.86	100.00	16
Canine distemper virus strain 13_2262	KJ747372.1	USA	Archive strain	96.67	100.00	17
Canine distemper virus genomic RNA	AB474397.1	Japan	lymph node	96.67	100.00	17
Canine distemper virus isolate SE/1994	KU578257.1	Germany	isolate="SE	96.47	100.00	18

DISCUSSION

CDV is one of the most serious infectious diseases found in dogs. It has a global distribution, which often affects young dogs that are not vaccinated; it has been observed in vaccinated puppies, and cases in other genetically affected hosts (12). In the present work, vaccinated and non-vaccinated dogs showed to have a risk for transmitting infectious diseases because of insufficient vaccination programs and quarantine steps in many breeding kennels and animal shelters in Iraq ; also the enormous genetic variety of prevalent CDV strains, substantial antigenic variations may be existed, resulting in vaccination escape instances (13–15). Quarantine procedures and reliable vaccine protocols had not been compulsory in the Iraqi animal shelters where the dogs resided.

The diagnosis of distemper often necessitates laboratory examinations to validate disease in the absence of usual symptoms and clinical signs that are close to those of other illnesses. One-step reverse transcription PCR is proved to be a sensitive and practical tool for detecting CDV in diagnostic laboratories (16). In the current research, CDV was detected by the N gene-based RT-PCR in 26% of the tested samples, indicating that the virus was circulating in the study region and because that, only animal with CDV clinical signs were taken a sample from it, which cannot be considered a reliable predictor of CDV prevalence. In previous research, RT-PCR revealed a 46.6 percent CDC positivity rate infection, which is higher than the positivity rate infection found in this study (17,18). The presence of CDV was also confirmed in the samples by DNA sequencing of the CDV's N gene (18,19).

The CDV negative samples which was detected by one-step RT-PCR, could indicate that the sick dog has a limited volume of viral load such as initial stages or late CD or convalescence period. As a result, nested PCR could be suggested for re-amplification of a portion of the CDV NP gene by RT-PCR (20).

The results of phylogenetic tree construction of the local Iraqi CDV virus isolates were found to be closely related to NCBI-Blast reference CDV isolates, including FJ977579.1 China, AF378705 USA, and AF305419.1 UK. Others were determined to be distinct, including EU072200.1 Hungary, AF164967.1 Switzerland, KU578257.1 Germany, and AB474397.1 Japan. The current research demonstrated how these strains can spread through the Iraq areas by importing the infected domestic dogs from different countries. These results are similar to many studies that have proven the major role of transmitting the disease by importing infected animals to Iraq (21).

Multiple Sequence Alignment of the Iraqi isolate revealed higher level of similarity with the "Snyder Hill" CDV and Onderstepoort CDV strains. Several reports have shown that vaccine strains are excreted following CDV vaccination because these strains are used in the production of vaccines (22). This was observed in the present study where the CDV PCR-positive dogs were infected with the vaccine strain of this virus.

The study confirmed that CDV infection is present in domestic dogs in Iraq. This may indicate a risk of the disease spreading to parts of the country that may be disease-free. Regular vaccination programs should be conducted on a consistent basis to decrease the possibility of introducing contagious pathogens to Iraq by importing free dogs with complete vaccination, and dog owners must be informed by an educational program planed by the authorities regarding the importance of a full vaccine program for domestic dogs.

ACKNOWLEDGEMENTS

The researchers would like to thank the staff at Baghdad Veterinary Hospital for the initial diagnosis and sample collection, as well as the staff at the diagnostic laboratory at the College of Veterinary Medicine, University of Baghdad.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Cho HS, Park NY. Detection of canine distemper virus in blood samples by reverse transcription loop-mediated isothermal amplification. J Vet Med Ser B Infect Dis Vet Public Heal. 2005; 52(9): 410–413.
2. Appel MJG, Summers BA. Pathogenicity of morbilliviruses for terrestrial carnivores. Vet Microbiol. 1995; 44(2–4): 187–191.
3. Krakowka S, Cockerell G, Koestner A. Effects of canine distemper virus infection on lymphoid function in vitro and in vivo. Infect Immun. 1975; 11(5): 1069–1078.
4. Zhao J, Shi N, Sun Y, Martella V, Nikolin V, Zhu C, et al. Pathogenesis of canine distemper virus in experimentally infected raccoon dogs, foxes, and minks. Antiviral Res. 2015; 122: 1–11.
5. Alldinger S, Baumgärtner W, van Moll P, Örvell C. In vivo and in vitro expression of canine distemper viral proteins in dogs and non-domestic carnivores. Arch Virol. 1993; 132(3–4): 421–428.
6. Appel MJG, Barr M, Pearce-Kelling S, Summers BA, Yates RA, Santinelli S, et al. Canine distemper epizootic in lions, tigers, and leopards in North America. J Vet Diagnostic Investig. 1994; 6(3): 277–288.
7. Al-Azawy AK, Al-Ajeeli KS, Ismail A. Isolation and identification of wild isolate of Newcastle disease virus from broiler farm in Diyala province: virological and histopathological study. Iraqi J. Vet. Med. 2019; 42(2): 41–49.
8. Blixenkrone-Møller M, Pedersen IR, Appel MJ, Griot C. Detection of IgM antibodies against canine distemper virus in dog and mink sera employing enzyme-linked immunosorbent assay (ELISA). J Vet Diagn Invest. 1991; 3(1): 3–9.
9. Guy JS. Diagnosis of canine viral infections. Vet Clin North Am Small Anim Pract. 1986; 16(6): 1145–1156.
10. Frisk AL, König M, Moritz A, Baumgärtner W. 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. 1999; 37(11): 3634–3643.
11. Bolívar P, Céspedes PF, Navarro C. Use of the reverse transcription-polymerase chain reaction for differential detection of two lineages of the canine distemper virus in Chile. Insights Vet Sci. 2019; 3(1): 005–013.
12. Horzinek MC. Vaccine use and disease prevalence in dogs and cats. Vet Microbiol. 2006; 117(1): 2–8.
13. Roth JA, Spickler AR. Duration of immunity induced by companion animal vaccines. Anim Health Res Rev. 2010; 11(2): 165–190.
14. Schultz RD, Thiel B, Mukhtar E, Sharp P, Larson LJ. Age and long-term protective immunity in dogs and cats. J Comp Pathol. 2010; 142(Suppl 1): S102–S108.
15. Riley MC, Wilkes RP. 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. 2015; 12(1): 1–10.
16. Sambade A, Martín S, Olmos A, García ML, Cambra M, Grau O, et al. A fast one-step reverse transcription and polymerase chain reaction (RT-PCR) amplification procedure providing highly specific complementary DNA from plant virus RNA. J Virol Methods. 2000; 87(1–2): 25–28.
17. Kim YH, Cho KW, Youn HY, Yoo HS, Han HR. Detection of canine distemper virus (CDV) through one step RT-PCR combined with nested PCR. J Vet Sci. 2001; 2(1): 59–63.
18. Sarute N, Delgado MV, Carrau L, Benech A, Francia L, Pérez R, et al. First genome sequence of a canine distemper virus strain from South America. Genome Announc. 2014; 2(5): e01009-14.
19. Shin Y, Cho K, Cho H, Kang S, Kim H, Kim Y, et al. Comparison of one-step RT-PCR and a nested PCR for samples. Aust Vet J. 2004; 82: 83–86.
20. Di Francesco CE, Di Francesco D, Di Martino B, Speranza R, Santori D, Boari A, et al. Detection by hemi-nested reverse transcription polymerase chain reaction and genetic characterization of wild type strains of Canine distemper virus in suspected infected dogs. J Vet Diagn Invest. 2012; 24(1):107–115.
21. Sawatsky B, Cattaneo R, von Messling V. Canine distemper virus spread and transmission to naive ferrets: selective pressure on signaling lymphocyte activation molecule-dependent entry. J Virol. 2018; 92(15): 1–13.
22. da Fontoura Budaszewski R, Streck AF, Nunes Weber M, Maboni Siqueira F, Muniz Guedes RL, Wageck Canal C. Influence of vaccine strains on the evolution of canine distemper virus. Infect Genet Evol. 2016; 41: 262–269.

الكشف الجزيئي عن فيروس طاعون الكلاب في مدينة بغداد، العراق

خليفة علي منصور[1]، سليم امين حسو[2]

[1]فرع فرع الطب الباطني والوقائي، كلية الطب البيطري، جامعة القادسية، العراق، فرع الطب الباطني والوقائي، كلية الطب البيطري، جامعة بغداد، العراق

الخلاصة

يعد مرض الطاعون (CD) مرضًا فيروسيًا شديد العدوى ومميتًا يصيب الكلاب، مع ارتفاع معدل الامراضية و الوفيات. يصيب فيروس طاعون الكلاب مجموعة واسعة من الحيوانات ،بما في ذلك العديد من لقرود والحيتان، والحيوانات آكلة اللحوم المختلفة، مما يؤدي الى اصابة العديدة من اجهزة الجسم .تم تشخيص الجين (N) العائد للحامض الرايبوسومي في 48 عينة دم تم اخذها من حيوانات التي يشك في اصابتها سريريا بطاعون الكلاب في مستشفى بغداد التعليمي. تم تضخيم قطعة من الجين (N) ذات طول قاعدة (591 bp). تم ارسال نتائج التضخيم للتسجيل في قاعدة بيانات المركز العالمي لمعلومات التكنولوجيا الحيوية وكانت اعداد الانضمام (MW682324.1, MW682325.1, and MW682326.1) . .العزلات العراقية الثلاثة اظهرت تشابه كبير مع العتر العالمية في قاعدة بيانات NCBI(FJ977579.1, AF378705 and AF305419.1.) بينما اظهرت بعض الاختلاف مع (EU072200.1, AF164967.1, KU578257.1 and AB474397.1) كذلك اظهرت تشابه بنسب عالية مع العتر "Onderstepoort"و "Snyder" Hill" بينما هناك تشابه بنسب قليلة مع العتر "A75/17" و"007Lm".تم الاستنتاج بان تقنية تفاعل سلسلة البلمرة المنعكس ذات الخطوة الواحدة ذات حساسية عالية وسرعة في تشخيص فيروس الطاعون.

الكلمات المفتاحية: فيروس طاعون الكلاب، تحري، تفاعل سلسلة البلمرة المنعكس، الشجرة الوراثية، جين النيوكليوكابسيد