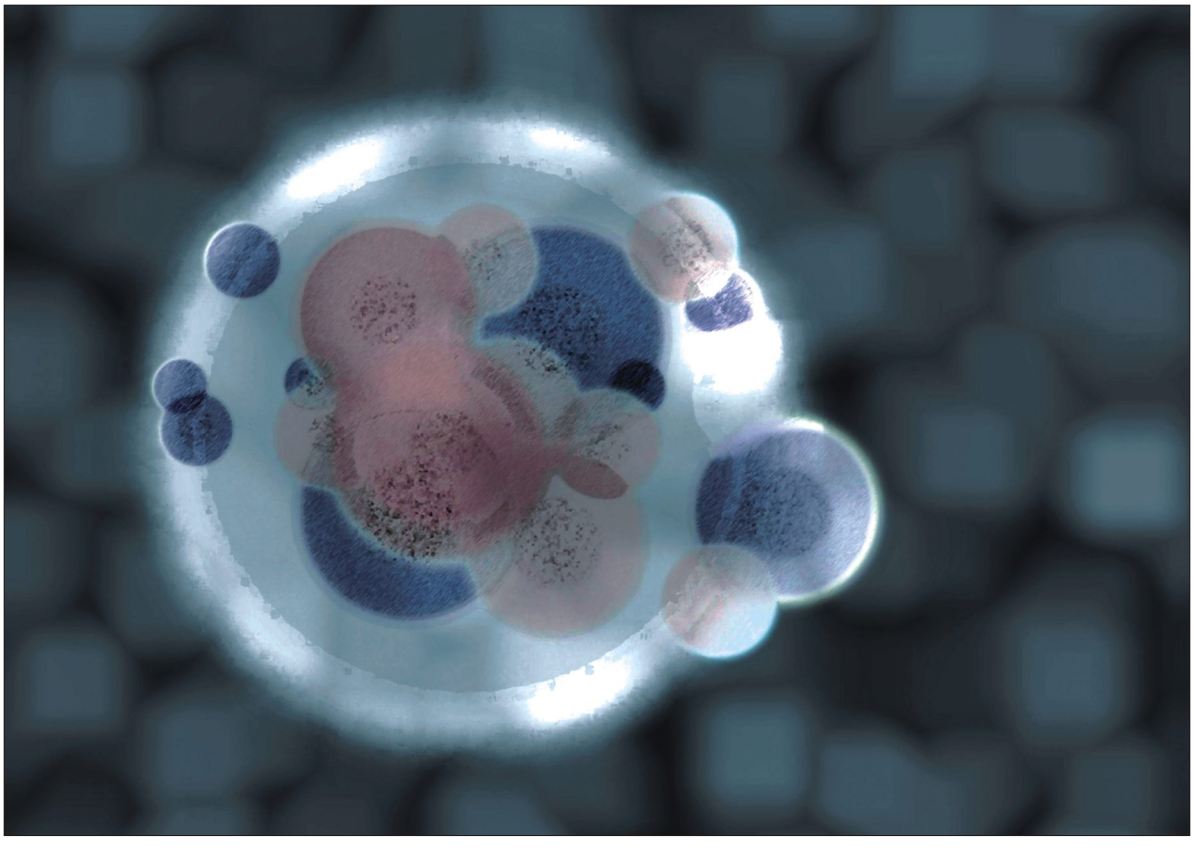

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2019.192.200

Research Article
Prevalence of Canine Parvovirus Infection in Egypt: Reliability of Some Molecular Methods Used for its Diagnosis in Dogs

[1]Romane A. Awad, [2]Ashraf G. Attallah and [3]Wagdy K.B. Khalil

[1]Department of Parasitology and Animal Diseases, Veterinary Division, National Research Center, 33 Bohouth St., 12622, Dokki, Giza, Egypt
[2]Department of Microbial Genetics, National Research Center, 33 Bohouth St., 12622, Dokki, Giza, Egypt
[3]Department of Cell Biology, National Research Center, 33 Bohouth St., 12622, Dokki, Giza, Egypt

Abstract

Background and Objective: Canine parvo enteritis is a highly fatal infectious disease of great importance in dogs, caused by canine parvovirus (CPV-2) which mutated during last 3 decades. The new strains CPV-2 a, CPV-2b and CPV-2c have been isolated globally, resulting in about 100% mortalities among infected dog populations. These great mortalities lead to major epidemiological problem. Therefore, the aim of this study was to investigate the prevalence of the disease, isolation, identification of causative virus and evaluation of different tests used for its diagnosis. **Materials and Methods:** About 189 dogs showing signs suggestive for CPV infection were subjected to clinical examination; rapid immunochromatography (IC) on fecal samples; conventional PCR on blood and faeces to detect CP viral DNA and to differentiate detected viral strains; quantitative RT-PCR to detect VP2 gene expression of viral cDNA; statistical analysis to study effect of age, sex and breed using Chi-square test and to evaluate sensitivity, specificity and accuracy of used tests and prevalence of CPV enteritis. **Results:** About 76 of 189 dogs showed clinical signs were positive for IC. About 87 samples were positive samples for CPV-2b (VP2) gene by nucleotide sequence analysis. About 105 positive samples detected by qRT-PCR. Statistical comparison of IC and conventional PCR to qRT-PCR showed that IC values were 72.38, 100, 84.65, 100 and 74%, PCR values were 82.85, 100, 90.47, 100 and 82.35% for sensitivity, specificity, accuracy, PPV, negative PV, respectively. No effect of sex, age, while, breeds showed effect on IC and PCR results. The prevalence of CPV infection was 55.56% among examined population. **Conclusion:** The results of the sequence analysis proved that PCR products of the CPV-2b cDNA had very low variations in their nucleotide sequence compared to published CPV-2b (VP2) gene. The gene sequence of CPV-2b strain in this study had deposited under the Ac. No: KY655746.1 in Gen-Bank. These results were confirmed by qRT-PCR assay quantitatively and qualitatively.

Key words: Canine parvovirus, CPV, qRT-PCR, sequencing, dogs, prevalence

Received: November 12, 2018 Accepted: December 21, 2018 Published: January 15, 2019

Citation: Romane A. Awad, Ashraf G. Attallah and Wagdy K.B. Khalil, 2019. Prevalence of canine parvovirus infection in Egypt: Reliability of some molecular methods used for its diagnosis in dogs. J. Biol. Sci., 19: 192-200.

Corresponding Author: Romane A. Awad, Department of Parasitology and Animal Diseases, Veterinary Division, National Research Center, 33 Bohouth St., 12622, Dokki, Giza, Egypt Tel: 00201223578982

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Canine parvovirus enteritis is highly contagious disease affects dogs from age of weaning and all ages. The disease clinically characterized by anorexia, depression, dehydration, pyrexia, vomiting, diarrhea which is profuse mostly hemorrhagic and leucopenia. Sometimes dyspnea or sudden death due to myocarditis which is more common in puppies of 4-6 weeks old[1-4]. Infection occur via the fecal-oral route and causative virus targeting cells of high mitotic activity in intestinal villi, lymphoid tissues and bone marrow[1-4].

Canine parvovirus viral enteritis caused by canine parvovirus CPV which designed as CPV type 2 (CPV-2) while CPV -1 is recorded as asymptomatic[5]. CPV-2 is a parvoviridae family member, autonomous group, single stranded DNA small virions, 28 nm in diameter, un-enveloped[6]. CPV-2 rapidly mutated to different new version strains designed as CPV- 2 a, CPV-2b, CPV- 2c which distributed worldwide[3,7].

In spite of the differences in genes and amino acids sequences among Autonomous group members (FPV, CPV-2a, CPV-2b,CPV-2c) are small, occurring mostly in VP2 gene which responsible for major capsid protein of these viruses[6,8,9].

Lack of maternal immunity and vaccination or incomplete vaccination programmes with modified live attenuated vaccines make puppies at high risk[2,3]. Recovery rate in diseased dog increases if dehydration, electrolytes imbalance and secondary bacterial infection overcomed[2,3].

No data regarding CPV-2 diagnosis by molecular identification and characterization had published. Rapid diagnosis, molecular detection of the causative virus is of great epidemiological importance for diagnosis and control of CPV-2 infection in certain countries where CPV is of great spread[3,7] plus facilities against virus treatment is low as in Egypt. Established routine diagnosis includes clinical examination, direct IC for qualitative detection of viral antigen in faeces has many disadvantages[3,7]. The current work aimed to achieve accurate detection using molecular methods for identification and genetic characterization of CPV-2 from different sources as blood and faeces from live naturally infected dogs. In addition to phylogenetic analysis was done for knowing the origin of CPV infection in Egypt.

MATERIALS AND METHODS

Duration of the study: This study has been conducted during the period between January 1, 2017 and October 30, 2018.

Ethical approval and informed consent: The team 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. The proposal of this study had approval from National Research Center comity no: 1/12/2016.

Chemicals: For molecular analysis, Trizol was bought from Invitrogen (Carlsbad, CA, USA). The revere 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 dogs: About 189 diseased dogs from different ages, sex and breeds were checked clinically for detection of clinical signs suggestive for CPV enteritis.

Sampling: Fecal samples were collected from all clinically infected cases (n = 189 dogs) and checked by rapid IC test (rapid CPV Ag test kit for qualitative detection of viral antigen in feces of all examined cases) and virus detection[8-11]. For detection of CP virus in blood and fecal samples were collected from all infected 189 dogs[8-11]. These dogs presented clinical signs of canine parvovirus enteritis. Collected samples were stored in 2 mL microtubes at a temperature of -80°C.

Clinical examination: One hundred and eighty-nine dogs 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[1,2]. 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 mild, moderate and severe[1-3,8].

Immune-chromatography (IC): Direct IC (the antigen rapid CPV Ag test kit, Bionote Inc., Korea) for the qualitative detection of Canine Parvoviral antigen in Canine feces was carried out on 189 fecal samples of dogs showing clinical signs of CP viral infection[9-11].

Molecular study

Extraction of total RNA and cDNA synthesis: Blood and fecal samples collected from naturally infected dogs were used to extract the total RNA using TRIzol® Reagent (Invitrogen, Germany) Kit. The isolation method was carried out according to the manufacturer's instructions of the above Kit. Approximately 500 µL from blood and 50 mg of fecal sample 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.

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 photo spectro metrically at 260 nm. 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 Revert AidTM 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[9-15].

Primer design: Specific primers used in this study were designed for different regions of VP2 gene which codes virus capsid protein using primer 3 program as illustrated in Table 1.

PCR amplification reaction: Three primer pairs which encoding CPV-2a, CPV-2b and CPV-2c were designed[9,12-15]. These primers were selected from different regions of VP2 gene were used in this study. The sequences of primer pairs are presented in Table 1.

The resultant cDNA was amplified by Eppendorf thermocycler using selected primers. Amplifications were performed with the PCR conditions. The PCR products were electrophoresed on 1.5% agarose gel and then were stained with ethidium bromide and visualized by Gel Doc., Bio-RAD, USA[12-15].

Sequence and phylogenetic analyses: The positive PCR products for CPV (RNA; DNA parvovirus) were selected and purified using an QIAGEN purification kits (QIAGEN, Germany). Sequencing was carried out based on the amplified segment using a standard ABI Big Dye terminator version 3.1 sequence kit (Applied Biosystem). The obtained sequences were analyzed for homology using the NCBI Basic Local Alignment Search Tool (BLAST: http://www.ncbi.nlm.nih.gov). Multiple sequence alignment was carried out using ClustalW2 and the percentage nucleotide identity was determined using DNA identity matrix. A neighbour-joining (NJ) phylogenetic tree was constructed based on the Canine parvovirus sequences using MEGA5 software. The tree reliability was assessed using 50 bootstrap replicates. All nucleotide sequences were deposited with the NCBI GenBank (Table 1)[16-20].

Table 1: CPV2-primers used in these study

Type of CPV2	Primer name	Oligonucleotide sequence (5'-3')	Estimated product size[a] (bp)
CPV-2a	F	CATTGGGCTTACCACCATTT	209
	R	AAATGGCCCTTGTGTAGACG	
CPV-2b	F	ATTATTTGTGAAAGTTGCGCCT	229
	R	TGGATTCCAGTATGAGAGGCTC	
CPV-2c	F	CATTGGGCTTACCACCATTT	209
	R	AAATGGCCCTTGTGTAGACG	
GAPDH	F	GAGAAAGCTGCCAAATATG	193
	R	CCAGGAAATGACCTTGACA	

[a]Based on available CPV genome sequences, CPV: Canine parvovirus

Quantitative real time-PCR (qRT-PCR): A step one real-time PCR system (Applied Biosystem, USA) was used to assess the copy of the cDNA of blood and fecal samples to detect the expression values of the canine parvovirus VP2 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 parvovirus gene (CPV-2a, CPV-2b and CPV-2c) were used (Table 1). The relative quantification of the target genes to the reference (GAPDH,)[21] was determined by using the 2-ΔΔCT method.

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, PPV and negative predictive values of used diagnostic tests and prevalence of the disease in population of this study were calculated according to Smith[22].

RESULTS

Clinical examination: The severity of the disease varied from severe, acute to subacute form that was fatal usually in puppies. Clinical signs were summarized in Table 2. Some of the clinical signs of the disease encountered in infected dogs in this study.

Table 2: Clinical signs detected in examined dogs

No. of cases	Breed		Age (month)	Sex		Lethargy	Fever (°C)	Anorexia	Thirst	Vomiting	Diarrhea	Dehydration (%)	Mucous membranes
	Small*	Large**		Male	Female								
76	-	76	1.5-2	36	40	Severe	40	Severe	Severe	Severe	Severe	10-12	Pale
38	8	30	2-4	20	18	Severe	40	Severe	Severe	Severe	Severe	10-12	Pale
33	3	30	4-6	15	18	Severe	39.5- 40	Severe	Severe	Severe	Severe	6-8	Pale
32	2	30	6-8	15	17	Moderate	39.5- 40	Severe	Severe	Moderate	Moderate	6-8	Pale
10	-	10	8-12	5	5	Mild	38-39	Severe	Severe	Mild	Mild	5-6	Pale

*Small breeds includes: Bull dog, Chihuahua, Chinese crusted, **Large breeds includes: Doberman, German shepherd, Rottweiler, Pitbull, Golden Retriever, Labrador Retriever, Siberian Husky, Caucasian dog and Saint Bernard breed

Table 3: Results of examined samples for CPV using Immune-chromatography

Case	N (%)	
	Male	Female
Positive	36 (39.5)	40 (40.8)
Negative	55 (60.4)	58 (59.1)
Total	91	98

Table 4: Results of conventional PCR of examined samples

Case	N (%)	
	Male	Female
Positive	41 (45.1)	46 (46.9)
Negative	50 (54.9)	52 (53.1)
Total	91	98

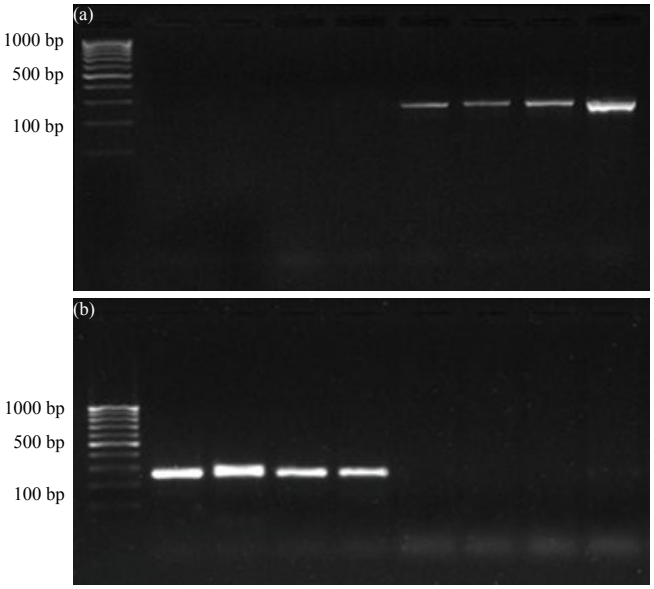

Fig. 1(a-b): Positive and negative dog samples identified by CPV-2b (VP2) gene (229 bp), (a) Feces samples and (b) Blood samples

Immune-chromatography (IC): Results of IC showed that 76 fecal samples from 189 of examined dogs were positive, results were distributed between males and females as shown in Table 3.

Molecular study

Conventional PCR: The PCR succeeded in identifying 87 infected cases among 189 examined dog samples as shown in Table 4.

Template cDNA collected from dog samples (blood and feces) with clinical signs of Canine parvovirus was amplified using the specific CPV primers that was previously designed according to complete CDS of capsid protein gene of CPV genome. In the present study, 3 specific primers were used encoding CPV-2a, CPV-2b and CPV-2c analogues.

The cDNA from all examined CPV samples produced clear bands upon amplification with its primer with a 229 bp product size (Fig. 1). The primer was specific for CPV-2b (VP2) gene fragment in infected dogs. However, the other analogues including CPV-2a and CPV-2c were not identified in any analyzed samples as well as negative dogs exhibited no CPV bands.

Sequencing results: Analysis of the genomic region encompassed by the primers of the CPV-2b gene and its predicted amino acid sequence allowed discrimination of CPV-2b from all its variants. Analysis of the sequences of all products illustrated in phylogenetic tree showed a high degree of nucleotide homology. The identification of the CPV-2b sequence in the current study was determined according to BLASTN programs version 2.5.1+at the GenBank NCBI on the website http://blast.ncbi.nlm.nih.gov/Blast.cgi, using FASTA format. The assembled sequence was assigned by Gene Bank accession numbers KY655746.1 and revealed that the examined sequence significantly matched CPV-2b, with a maximum of 99.8% identity with the VP2 gene sequences of the reference strain (accession number = HG004610) available in the Gene Bank. Phylogenetic analyses of isolates with other sequences are depicted in Fig. 2.

The sequence of CPV-2b identified in this study and the full sequences of the CPV-2b gene (published isolates collected from various parts of the world, including Asia, Europe, South America, North America and Australia from

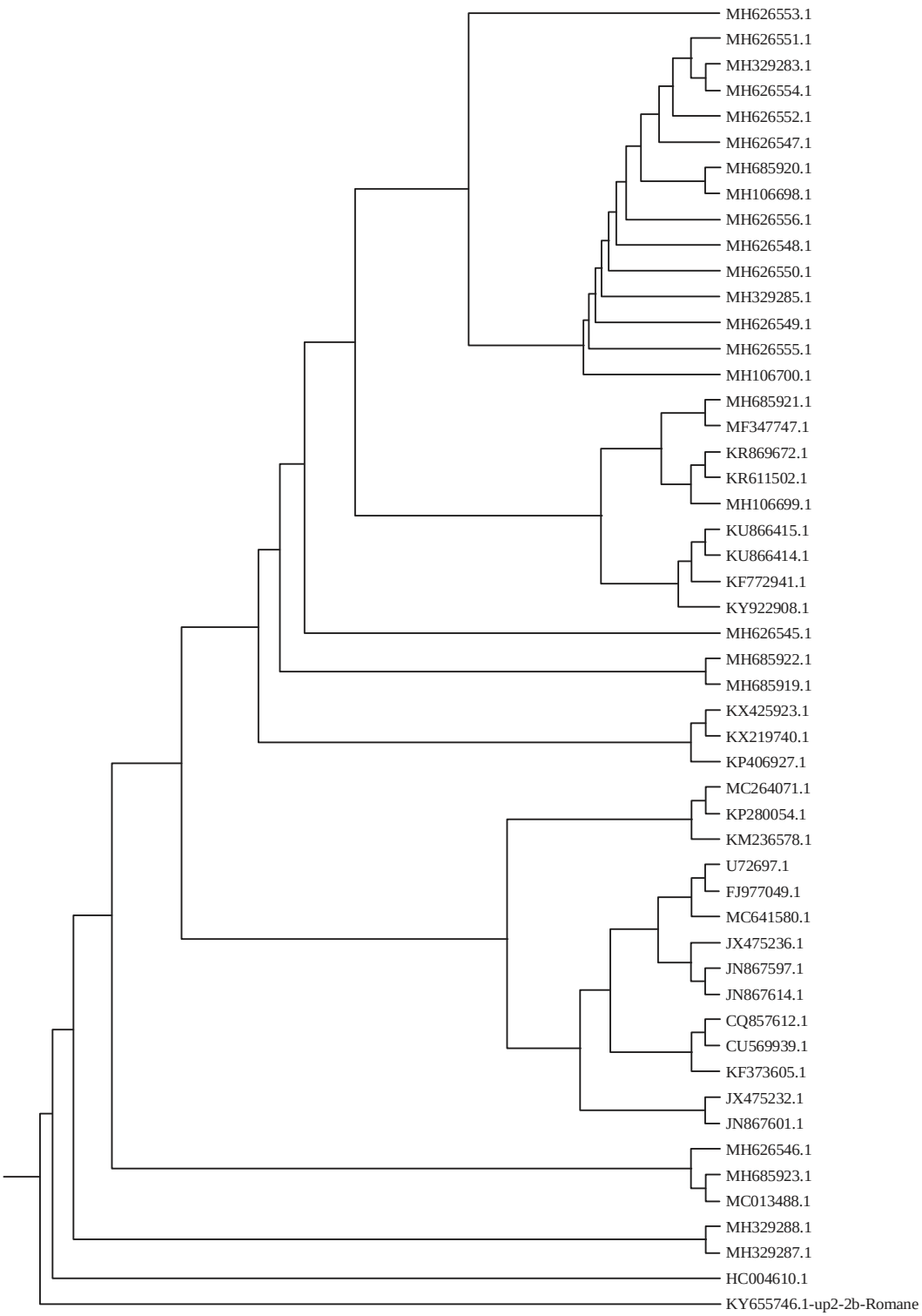

Fig. 2: Phylogenetic tree constructed from the CPV-2b gene nucleotide sequences of the Canine parvovirus strain generated in this study and other sequences obtained from the GenBank database

GenBank) were phylogenetically analyzed with the MEGA version 4.0 software (Fig. 2). The data showed that several different clusters were formed, the sequence isolated in this study in a distinct clade showed more similarity and closely related to strain Canine Parvo Virus-2b H in C004610.1from India (99.8% similarity).

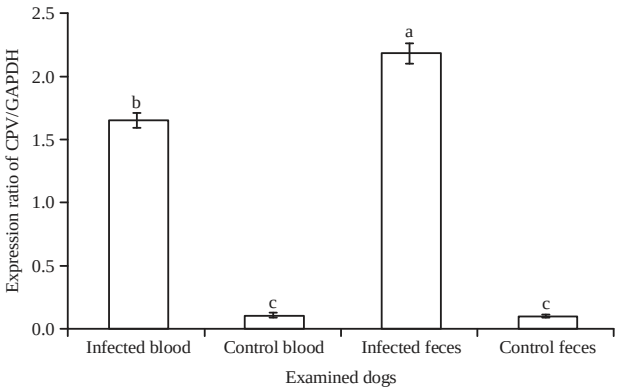

Fig. 3: Expression levels of CPV-2b (VP2) gene in different dog samples

Data are presented as Mean ± SEM, [a,b,c]followed by different superscripts are significantly different ($p \leq 0.05$)

Table 5: Comparison between immune-chromatography (IC) according qRT-PCR findings of examined cases of CPV

	IC		
Method of identification	Positive	Negative	Total
qRT-PCR			
Positive	76 (T+)	29 (F-)	105
Negative	0 (F+)	84 (T-)	84
Total	76	113	189

T+: True positive, T-: True negative, F+: False positive, F-: False negative, Sensitivity: 72.38%, Positive pre-detective values (+PPV): 100%, Specificity: 100% negative pre-detective values, -NPV: 74%, Accuracy: 84.65%

Table 6: Comparison between qRT-PCR and conventional PCR and findings of examined cases of CPV

	PCR		
Method of identification	Positive	Negative	Total
qRT-PCR			
Positive	87 (T+)	18 (F-)	105
Negative	0 (F+)	84 (T-)	84
Total	87	102	189

T+: True positive, T-: True negative, F+: False positive, F-: False negative, Sensitivity: 82.85%, Positive pre-detective values (+PPV): 100%, Specificity: 100%, negative pre-detective values (-NPV): 82.35% and Accuracy: 90.47%

Quantitative real time-PCR

Expression levels of CPV-2b in dogs: qRT-PCR succeeded in detection of 105 truly infected cases among the population of the current study than that detected by conventional PCR (84 cases) and IC (76 cases).

Figure 3 represented the results of CPV gene expression levels in dog samples (blood and feces).

The results revealed that the CPV-2b (VP2 gene) was very low and not detected in all control dog samples. However, expression levels of CPV gene in different infected dog samples were very high compared with control dogs. Additionally, the expression levels of CPV gene increased with highly significant ($p<0.01$) differences in feces samples compared with blood samples (Fig. 3).

Statistical analysis: Results of statistical analysis proved that sex and age have no significant effect on results of IC and conventional PCR. While, breed play role on the results of both tests where there is significance difference between small and large breeds on accuracy of both IC and conventional PCR. qRT-PCR is considered as gold standard of this study.

Table 5 and Fig. 4 represented the comparison between IC and qRT-PCR showing 72.38, 100, 84.65, 100 and 74% on sensitivity, specificity, accuracy, positive predictive value and negative protective value

Table 6 and Fig. 4 represented the comparison between conventional PCR and qRT-PCR showing 82.85, 100, 90.47, 100 and 82.35% on sensitivity, specificity, accuracy, positive predictive value and negative protective value.

Figure 5 represented the comparison of results of different tests as percentages. The prevalence of CPV enteritis was 55.56% detected among the present population.

DISCUSSION

The present work was conducted by using conventional PCR technique which was designed firstly to detect presence of CPV-2 and secondly to differentiate the different CPV-2 strains in the population samples of the present work according to which stated by Mohyedini *et al.*[9], Zhong *et al.*[10] and Tinky *et al.*[11].

The PCR was achieved by primers designed and custom synthesized based on nucleotide sequence of VP2 gene of CPV strains: CPV-2a, CPV-2b and CPV-2c as recommended by Mohyedini *et al.*[9], Zhong *et al.*[10] and Tinky *et al.*[11].

In order to knowing the existing parvovirus strains in district where this study applied. The current findings were 87 cases were identified as infected cases with CPV-2b by conventional PCR among 189 cases showing clinical signs suggestive for Canine parvovirus enteritis, this in correlation with Zhong *et al.*[10], Tinky *et al.*[11] and Cavalli *et al.*[13].

PCR finding revealed that CPV-2b is the main strain circulating in Egypt where CPV-2a, CPV-2c not recorded in population of this work correlated with that of Gagnon *et al.*[23].

Phylogenetic analysis was done and the current phylogenetic tree among the genetically closet 50 CPV-2 isolates showing 98-100% genetic assembly with the sequence of VP2 in the current isolated strains partially (Accession no of the present study: KY655746.1-VP2-Romane 0.18856). VP2 in the isolated strains of this study was genetically stasis to VP2 gene of 50 CPV isolates included in this tree[18-20,23-25].

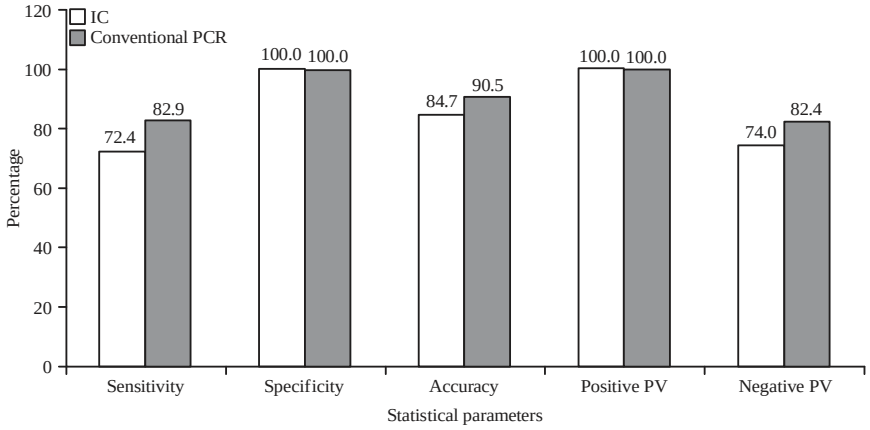

Fig. 4: Comparison of IC and conventional PCR according to the percentage of sensitivity, specificity, accuracy, positive and negative PV

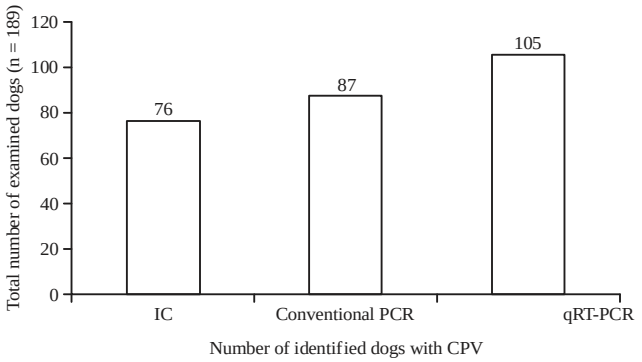

Fig. 5: Comparison between results of different assays for CPV-2b identification

This study proved that PCR technique is more accurate in detection of infected cases, PCR Showing 87 positive cases than IC test which detected 76 cases only this due to high sensitivity of conventional PCR in detection of infected cases even those harboring low log of viraemia as reported by Mohyedini *et al.*[9], Zhong *et al.*[10] and Tinky *et al.*[11].

PCR can differentiate between different CPV-2 strains, while this cannot achieved by IC which gave positive results only for infected cases carrying all strains CPV-2a, CPV-2b and CPV-2c, this in agreement with Mohyedini *et al.*[9] and Zhong *et al.*[10].

The only advantage of IC is its reliability for diagnosis of CPV infection in houses and veterinary clinics easily[9-11]. But IC test of moderate sensitivity so it detected only 76 infected dogs among 189 examined cases, where, these in correlation with Mohyedini *et al.*[9] and Faz *et al.*[26].

qRT-PCR due to its high accuracy and sensitivity[26] considered the gold standard test in this study to which conventional PCR and IC were compared according to Mohyedini *et al.*[9], Smith[22] and Faz *et al.*[26]. Conventional PCR showed 82.85, 100, 90.47, 100 and 82.35%, while IC showed 72.38, 100, 84.65, 100 and 74% for sensitivity, specificity, accuracy, positive pre-detective value and negative pre-detective value, respectively, these findings correlated with that of Faz *et al.*[26].

qRT-PCR (gene expression analysis test) in this study used as a qualitative accurate confirmatory test for detection of VP2 gene of CPV-2 strains in samples of infected cases[14-17].

Gene expression analysis test proved that it is not only a qualitative but also serve as molecular quantitative diagnostic technique with high accuracy value than conventional PCR for detection of low concentration of CPV-VP2 gene in fecal samples of infected dogs which may reach to 1.0-10^3-7.0$\times 10^9$ viral DNA in microgram of feces in agreement with Kumar and Nandi[12].

Also, qRT-PCR showed higher sensitivity in quantitative detection of CPV DNA than conventional PCR even in very low concentration which reach to 1-10^3 viral DNA in microgram of feces[12,17].

Prevalence of CPV enteritis in population of this study was 55.56% in Egypt, this findings are in correlation with that of Faz *et al.*[26], Miranda *et al.*[27] and Pedroza-Roldan *et al.*[28].

Statistical analysis results proved that there is no significant difference between male and females dogs fecal samples when assayed by either IC or PCR. By another mean sex has no effect on results of IC and PCR when used for diagnosis of CPV infection as confirmed by Faz *et al.*[26]. Age has no significant effect on results of IC and PCR . Thus, the IC and PCR have the same accuracy for all different age groups. These findings in correlation with that reported by Awad *et al.*[29], who, stated that both age and sex of infected dogs has no effect on results.

Statistically, the results found that there is a significant difference between small sized and large sized breed on accuracy of IC and PCR. This means that this significant difference between different dog breeds attributed to difference in susceptibility for CPV infection as recorded by Hall *et al*.[2], Goddard and Leisewitz[3] and Decaro *et al*.[26], who reported that Doberman and Rottweiler breeds are more susceptible than other dog breeds.

CONCLUSION

Prevalence of CPV-2b infection was 55.56% among population of this study. IC, conventional PCR and qRT-PCR all together considered reliable tests for diagnosis of CPV-2 infection in dogs accurately. The results of the sequence analysis proved that PCR products of the CPV-2b cDNA had very low variation in their nucleotide sequence of all isolates in comparison published CPV-2b (VP2) gene. The gene sequence of CPV-2b strain in this study had deposited under the Ac. No: KY655746.1 in Gen-Bank. These results were confirmed by results of gene expression analysis test (RT-PCR) quantitatively and qualitatively.

SIGNIFICANCE STATEMENT

This study succeeded in identification of the existing strain of CPV which is CPV-2b that makes alarm and mortalities among dog population, in addition to estimating the prevalence of CPV infection in dogs in Egypt. This study will help the researchers to uncover critical area of leakage in diagnostic abilities of IC and PCR in comparison to qRT-PCR, that not explored by many scientists. Thus, an clinico-epidemiological evaluation of IC and conventional PCR may be arrived at. The present results recommend the use of qRT-PCR as gold standard test with the use of different molecular methods in diagnosis and measuring CPV prevalence.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Brit Martens and Prof. Safwat Ali Hassan, Small Animals Referral Hospital- Merkurring 50, 22143 Hamburg-Rahlstedt, Germany, for the scientific support regarding the epidemiological data about the parvovirus infection in Germany. Also, the authors are grateful for Prof. Dr. Sobhy Abd-Elshafy, at Parasitology and Animal Diseases Department, Veterinary Division, National Research Center, Giza, Egypt, for providing the technical and scientific consultant regarding statistical analysis of data. The present study received no financial support.

REFERENCES

1. Gaskell, R.M., M. Bennett, B. Tennant and K. Willoughby, 1996. Feline and Canine Infectious Diseases. Iowa State Press, Ames, IA., ISBN: 9780632034468, Pages: 190.
2. Hall, E., J.W. Simpson and D.A. Williams, 2005. BSAVA Manual of Canine and Feline Gastroenterology. 2nd Edn., Wiley, USA., ISBN: 9780905214733, pp: 188-190.
3. Goddard, A. and A.L. Leisewitz, 2010. Canine parvovirus. Vet. Clin. North Am. Small Anim. Pract., 40: 1041-1053.
4. Smith-Carr, S., D.K. Macintire and L.J. Swango, 1997. Canine parvovirus. Part I. Pathogenesis and vaccination. Compend. Continuing Educ. Pract. Vet. USA., 19: 125-133.
5. Lamm, C.G. and G.B. Rezabek, 2008. Parvovirus infection in domestic companion animals. Vet. Clin. N. Am.: Small Anim. Pract., 38: 837-850.
6. Acheson, N.H., 2011. Fundamentals of Molecular Virology. 2nd Edn., Wiley, USA., ISBN: 9781118213827, Pages: 500.
7. Miranda, C. and G. Thompson, 2016. Canine parvovirus: The worldwide occurrence of antigenic variants. J. Gen. Virol., 97: 2043-2057.
8. Decaro, N., C. Desario, M. Billi, E. Lorusso and M.L. Colaianni *et al*., 2013. Evaluation of an in-clinic assay for the diagnosis of canine parvovirus. Vet. J., 198: 504-507.
9. Mohyedini, S., S. Jamshidi, S. Rafati, G.R. Nikbakht, A. Malmasi, Y. Taslimi and H. Akbarein, 2013. Comparison of immunochromatographic rapid test with molecular method in diagnosis of canine parvovirus. Iran. J. Vet. Med., 7: 57-61.
10. Zhong, Z., L. Liang, J. Zhao, X. Xu and X. Cao *et al*., 2014. First isolation of new canine parvovirus 2a from Tibetan mastiff and global analysis of the full-length *VP2* gene of canine parvoviruses 2 in China. Int. J. Mol. Sci., 15: 12166-12187.
11. Tinky, S.S., R. Ambily, S.R. Nair and M. Mini, 2015. Utility of a rapid immunochromatographic strip test in detecting canine parvovirus infection compared with polymerase chain reaction. Vet. World, 8: 523-526.
12. Kumar, M. and S. Nandi, 2010. Development of a SYBR Green based real-time PCR assay for detection and quantitation of canine parvovirus in faecal samples. J. Virol. Methods, 169: 198-201.
13. Cavalli, A., C. Desario, I. Kusi, V. Mari and E. Lorusso *et al*., 2014. Detection and genetic characterization of Canine parvovirus and Canine coronavirus strains circulating in district of Tirana in Albania. J. Vet. Diagn. Invest., 26: 563-566.
14. Zhou, H., L. Liu, R. Li, Y. Qin and Q. Fang *et al*., 2017. Detection and genetic characterization of canine astroviruses in pet dogs in Guangxi, China. Virol. J., Vol. 14. 10.1186/s12985-017-0823-4.
15. Tu, M., F. Liu, S. Chen, M. Wang and A. Cheng, 2015. Role of capsid proteins in parvoviruses infection. Virol. J., Vol. 12. 10.1186/s12985-015-0344-y.

16. Da Rocha Gizzi, A.B., S.T. Oliveira, C.M. Leutenegger, M. Estrada and D.A. Kozemjakin *et al*., 2014. Presence of infectious agents and co-infections in diarrheic dogs determined with a real-time polymerase chain reaction-based panel. BMC Vet. Res., Vol. 10. 10.1186/1746-6148-10-23.
17. Streck, A.F., D. Ruster, U. Truyen and T. Homeier, 2013. An updated TaqMan real-time PCR for canine and feline parvoviruses. J. Virol. Methods, 193: 6-8.
18. Dogonyaro, B.B., A.M. Bosman, K.P. Sibeko, E.H. Venter and M. van Vuuren, 2013. Genetic analysis of the VP2-encoding gene of canine parvovirus strains from Africa. Vet. Microbiol., 165: 460-465.
19. Lin, Y.C., S.Y. Chiang, H.Y. Wu, J.H. Lin, M.T. Chiou, H.F. Liu and C.N. Lin, 2017. Phylodynamic and genetic diversity of canine parvovirus type 2c in Taiwan. Int. J. Mol. Sci., Vol. 18. 10.3390/ijms18122703.
20. Kaur, G., M. Chandra and P.N. Dwivedi, 2016. Phylogenetic analysis of VP2 gene of canine parvovirus and comparison with Indian and world isolates. Acta Virol., 60: 106-110.
21. Tarlinton, R.E., H.K.R. Barfoot, C.E. Allen, K. Brown, R.J. Gifford and R.D. Emes, 2013. Characterisation of a group of endogenous gammaretroviruses in the canine genome. Vet. J., 196: 28-33.
22. Smith, R.D., 2005. Veterinary Clinical Epidemiology. 3rd Edn., CRC Press, Boca Raton, ISBN: 9780849315664, Pages: 280.
23. Gagnon, C.A., V. Allard and G. Cloutier, 2016. Canine parvovirus type 2b is the most prevalent genomic variant strain found in parvovirus antigen positive diarrheic dog feces samples across Canada. Can. Vet. J., 57: 29-31.
24. Decaro, N., C. Desario, D.D. Addie, V. Martella and M.J. Vieira *et al*., 2007. Molecular epidemiology of canine parvovirus, Europe. Emerg. Infect. Dis., 13: 1222-1224.
25. Kaur, G., M. Chandra, P.N. Dwivedi and N.S. Sharma, 2014. Antigenic typing of canine parvovirus using differential PCR. VirusDisease, 25: 481-487.
26. Faz, M., J.S. Martinez, I. Quijano-Hernandez and R. Fajardo, 2017. Reliability of clinical diagnosis and laboratory testing techniques currently used for identification of canine parvovirus enteritis in clinical settings. J. Vet. Med. Sci., 79: 213-217.
27. Miranda, C., J. Carvalheira, C.R. Parrish and G. Thompson, 2015. Factors affecting the occurrence of canine parvovirus in dogs. Vet. Microbiol., 180: 59-64.
28. Pedroza-Roldan, C., V. Paez-Magallan, C. Charles-Nino, D. Elizondo-Quiroga, R.L. de Cervantes-Mireles and M.A. Lopez-Amezcua, 2015. Genotyping of canine parvovirus in western Mexico. J. Vet. Diagn. Invest., 27: 107-111.
29. Awad, R.A., W.K.B. Khalil and A.G. Attallah, 2018. Epidemiology and diagnosis of feline panleukopenia virus in Egypt: Clinical and molecular diagnosis in cats. Vet. World, 11: 578-584.