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Clinical and Molecular Diagnosis of Parvovirus Infection in Household Dogs

Omar H. S. Qubaa[1], Mohammad A. Hamad[2]

[1.] M.Sc. student. Microbiology. Department of Microbiology-College of Veterinary Medicine, University of Mosul,A veterinarian in the Nineveh Agriculture Directorate.

[2.] Prof. Dr. Microbiology, Department of Microbiology-College of Veterinary Medicine, University of Mosul.

Abstract

Canine parvovirus is a serious famous virus in dogs worldwide, causing great danger to their health. The present study was carried out on 100 diseased dogs of different ages, breeds, and both sexes. All clinical symptoms were recorded and classified. Fecal samples were collected for two purposes, firstly for detection of parvovirus antigen using rapid agglutination test, and secondly for extraction and molecular diagnosis of the disease using PCR technique. The principal clinical signs that were recorded included: fever, loss of appetite, bloody diarrhea, vomiting, dehydration. These signs appeared together in almost all cases and the characteristic signs syndrome was involved fever + loss of appetite + Bloody diarrhea. The disease showed high distribution in males (81.3%), while the females were less infected (66.7%). The results of the study revealed a high incidence of the disease in dogs up to or less than 6 months of age (87.1%) whereas the clinical cases were least in older dogs (57.9%), and depending on the breed the most cases were in German shepherd (29%), Husky (14%), local breeds (12%) and others. The clinical cases were also classified according to the period of gathering during the study and the peak of cases was in December (23 cases) to January (29 cases). The techniques were used for the diagnosis of the disease displayed high accuracy since rapid test results showed (68%) of cases were positive for CPV, and PCR results were higher than that rate (76%). Both couples of primers revealed the same results for the detection of CPV. In conclusion, CPV is a serious disease in household dogs in Mosul city and more dominant during winter, although the results of PCR were higher both techniques showed satisfactory results for the detection of CPV. It's recommended to diagnose the strains of CPV that distributing in Mosul city.

Keywords: CPV, Household dogs, PCR, Rapid

Introduction:

Viral enteritis is one of the most frequent causes of infectious diarrhea in young dogs. Canine parvovirus type 2 (CPV-2, CPV) is infecting dogs and has been implicated as a primary pathogen (Greene, 2006). It is considered the most common cause of puppy enteritis and death (Kapil, 1995). CPV has unique properties that make it an emerging and re-emerging pathogen in dogs worldwide and a serious cause of morbidity and mortality in young dogs since its discovery in 1978 (Appel *et al*., 1999; Hong *et al*., 2009). The virus's tendency to "reinvent" itself and

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transform into new, more virulent, and immune subspecies contributes to the virus's continued prevalence. (Goddard & Leisewitz, 2010).

CPV mainly has two antigenic types, type 2a (CPV-2a) and type 2b (CPV- 2b). In Italy, another antigenic variant type 2c (CPV-2c), was discovered. (Buonavoglia *et al*., 2001).CPV-2 infection affects dogs of both sexes, ages, and species. (Castro *et al*., 2007; Gombac *et al*., 2008).

The clinical cases suffer from fever, anorexia, nausea, fatigue, and mucoid or bloody diarrhea, followed by dehydration(Robinson *et al*., 1979). The mortality rate of those infected was 16-48% but reached 91% in untreated cases (Aiello *et al.,* 2006). Leukopenia may present leading to death occurring as short as two days after onset of disease. The neurological disease could resultfrom sepsis, electrolyte imbalances, hypoglycemia, and CNS hemorrhage due to the disseminated intravascular coagulation (Ettinger & Feldman 1995; Jones *et al*., 1997; Schwartzberg *et al,* 2002).Myocarditis may progress after utero-exposure or before the age of eight weeks. (Ettinger and Feldman, 1995; Greene, 1998).,About 25% of puppies were developed asymptomatic urinary tract infections Following parvovirus enteritis (Aiello *et al.*, 2006). Oronasal exposure to infected urine, hair coats, and fomites such as tools, mosquitoes, and rodents facilitate the spread of the disease. The virus will live for months or even years in the atmosphere.

Different techniques for detecting antibodies in the blood can be used to identify the CPV, which is particularly effective in the first 5-7 days of clinical symptoms(Rimmelzwann *et al*., 1991). Also, the rapid test can be used to diagnose canine parvovirus antigen in the fecal sample (AL-Bayati, 2010; Al-Tayib O., 2014). The molecular techniques are the accurate methods for the diagnosis of CPV and can be done directly on the fecal samples and swabs (Vikas Gupta et al.,2017;Baba Sheikhet al.,2017;Dastmalchi Saei et al.,2016). The main principles for diagnosis of CPV in household dogs depending on the clinical signs and some clinics use rapid test, therefore and also for little studies on this disease in the governorate, the current study aimed to diagnose CPV by molecular technique beside other methods and also relating the disease with age, breed, sex of dogs and also a correlation with the seasonal effect.

MATERIALS AND METHODS

Samples collection:

One hundred (100) fecal swabs and samples were collected from clinically diseased dogs of different ages, breeds, and both sexes, which were brought to private veterinary clinics (pet care clinic). All clinical symptoms were recorded and classified (Castro et al., 2007; Sara et al.,2006).

The fecal samples and swabs were collected for two purposes, firstly for detection of parvovirus antigen using rapid agglutination test, and secondly for molecular diagnosis that holds at -20°C until they were tested (Al-Tayib O., 2014).The clinical samples were collected between July 2020 and March 2021 and classified according to the period of collection (Jassim M.,2017).

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Rapid test application:

Rapid CPV Ag test Procedure

All reagents and samples were put at room temperature (15~30C) when used. A swab of the fecal sample was inserted into the assay diluents tube and the swab was mixed until the sample dissolved into the assay diluents (Approximately 10 seconds). Waited for 1 minute to settle down the large particles. The test device was removed from the foil pouch and placed on a flat and dry surface. Took the supernatant sample in the tube using the disposable dropper and added 4 drops of mixed sample into the sample hole, drop by drop vertically. test results were interpreted at 10 minutes (Figure 1).(Jassim M.,2017).

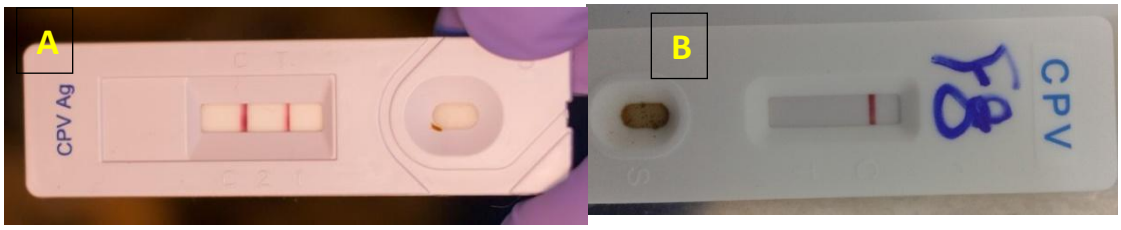

Figure (1): Rapid test kit for detection CPV antigen (A=positive results, B=negative results).

Extraction of the DNA:

DNA was extracted according to the manufacturer's instructions using a stool DNA extraction kit (QIAamp® Quick DNA Stool Mini Kit) (Figure 2).

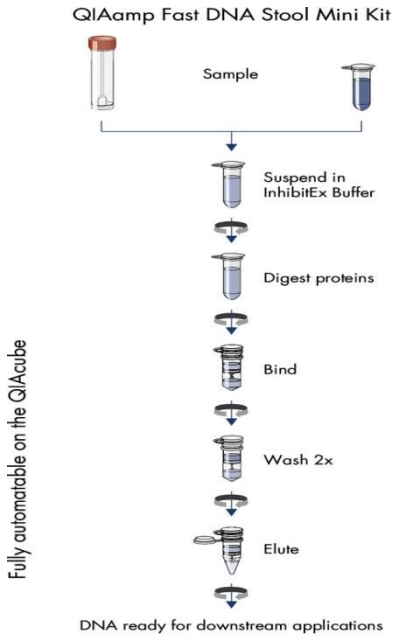

Figure (2): Extraction technique

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The purity and concentration of extracted DNA were measured by nanodrop spectrophotometer(Bio Drop-Micro-Volume Measurement Platforms, USA) in the Biology department-College of Sciences, University of Mosul.

Primers, reaction's solution, amplification programs, and electrophoreses:

Two couples of primers (IDT Inc., USA) were used for the detection of CPV (VP2 and PVP2 gens), each couple primers in a separate reaction (Table 1) (Buonavoglia et al.,2001; McEndaffer *et al*., 2017). The total volume of the reaction solution was (20 µL) and composed of template DNA (2µL), master mix (10 µL), 1.5 µL of each primer, and PCR water (5 µL).The Programs of amplification for each couple of primers were mentioned in table (2) (Buonavoglia *et al*., 2001; McEndaffer *et al*., 2017).

The results of amplification were detected by immigrated through 1.5% agarose gel, which was stained with ethidium bromide 0.5 g/mL and compared to DNA marker (100 base pair ladder), then visualized with a UV transilluminator.

Table 1: Primers sequences

Primers	The sequence of the primers (5- to 3-)	Reference
VP2-F	CAGGAAGATATCCAGAAGGA	Buonavoglia *et al*., 2001
VP2-R	GGTGCTAGTTGATATGTAATAAACA	
PVP2-F	TTACTAAGAACAGGTGATGAA	McEndaffer *et al*., 2017
PVP2-R	ATTTGGATAAACTGGTGGT	

Table 2: The Programs of amplification

Parvovirus VP2 (Buonavoglia *et al*., 2001)			
Step	**Temperature (℃)**	**Time**	**Number of Cycles**
Initial Denaturation	95	2 minutes	1 cycle
Denaturation	95	30 seconds	35 cycles
Annealing	50	30 seconds	
Extension	72	40 seconds	
Final Extension	72	10 minutes	1 cycle
PVP2 program (McEndaffer *et al*., 2017)			
Step	**Temperature (℃)**	**Time**	**Number of Cycles**
Initial Denaturation	95	10 minutes	1 cycle

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Denaturation	95	45 seconds	35 cycles
Annealing	50	45 seconds	
Extension	72	60 seconds	
Final Extension	72	5 minutes	1cycle

Results

The mainrecordedclinical signs include Fever (86%), loss of appetite (81%), Bloody diarrhea (62%), Vomiting (37%), Dehydration (31%) (Table3, figures 3-6). The high percentage of clinical cases suffered from 2-3 clinical signs at the same time, and most suffered from fever+ loss of appetite + Bloody diarrhea (43%) as the principal signs together, while the other mixed signs appeared at lesser rates (Table 4).According to the sex, the 100 clinical cases included 64% males and (36%) females. The high percentage of males (81.3%) appeared positive for the diagnosis of CPV by PCR, while (66.7%) of females showed the infection according to PCR (Table 5).Depending on the age groups, the clinical cases included 62 dogs with ages less than or up to 6 months, and 54 dogs out of 62 appeared positive for CPV (87.1%, according to the PCR results). While 38 infected dogs classified in ages more than 6 months and 22 (57.9%) dogs were appeared positive for CPV (Table 6).

For detection of CPV according to the breeds, 33 clinical cases were German shepherd, 19 cases of Husky, 17 local breeds, 16 Doberman pinschers, and 15 cases terrier (Table 7).The clinical cases were also classified according to the period of gathering during the study that included: October (13 cases), November (17), December (23), January (29), February (10), and March (8). Also, linked the collection period together with the detection by Rapid test and PCR and recorded the results (Table 8). Rapid test results showed 68 cases out of 100 clinical cases were positive for CPV and 32 cases negative (Table 9).

The purity and concentration of extracted DNA from fecal swabs and samples were ranged between 1.7 to 1.8 (purity) and 20 ng/µl (Concentration). The PCR results for both two couple primers (VP2, PVP2) showed that 76 (76%) cases out of the total cases were positive for CPV and the negative results appeared in 24(24%) clinical cases (Table 9, figure 7).

Fig.3: dogs with severe bloody diarrhea

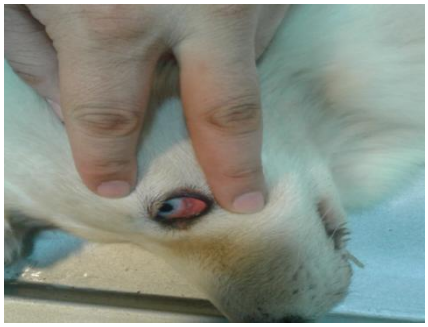

Fig.4: dogs with high fever

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Fig. 5: Depression with bloody diarrhea

Fig. 6: Dehydration

Table3:The recorded clinical signs

signs	(Number) of cases	Percent. %
Fever	86	86%
loss of appetite	81	81%
Bloody diarrhea	62	62%
Vomiting	37	37%
Dehydration	31	31%

Table 4: The recorded mixed clinical signs

Mixed signs	(Number)	Positive (Percent)
Fever+ Bloody diarrhea+loss of appetite	43	43%
Fever +vomiting	19	19%
Bloody diarrhea + fever + Dehydration+loss of appetite	17	17%
Vomiting+ Bloody diarrhea + Dehydration+ loss of appetite	14	14%
Fever +bloody diarrhea + loss of appetite+	7	7%
Total	100	100%

Table 5:Infection rates of CPV according to the sex.

sex	(Number)	Percentage	Positive to CPV	Percent. of positive	Percent. to all

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male	64	64%	52	81.3%	52%
Female	36	36%	24	66.7%	24%
Total	100	100	76	76%	76%

Table 6: The infection rates of CPV in different age groups.

Age group	Total	Positive (Number)	Percentage
6 months or less	62	54	87.1%
More than 6 months	38	22	57.9%
Total	100	76	76%

Table 7:The infection rate of CPV in various breeds

Breed	Number	Positive	Positive Percent.
German shepherd	33	29	29%
Husky	19	14	14%
Local breeds	17	12	12%
Doberman Pinschers	16	11	11%
terrier	15	10	10%
Total	100	76	76%

Table 8: The collected clinical cases according to the period

Clinical cases	Oct	Nov	Dec	Jan	Feb	March
Total cases	13	17	23	29	10	8
Raped test	8	11	16	21	7	5
PCR	9	13	18	23	7	6

Table 9: The comparison between results of rapid agglutination test and PCR

Samples	**Rapid test**		**PCR**	
	Positive	Negative	Positive	Negative
100	68	32	76	24

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Percentage	68%	32%	76%	24%

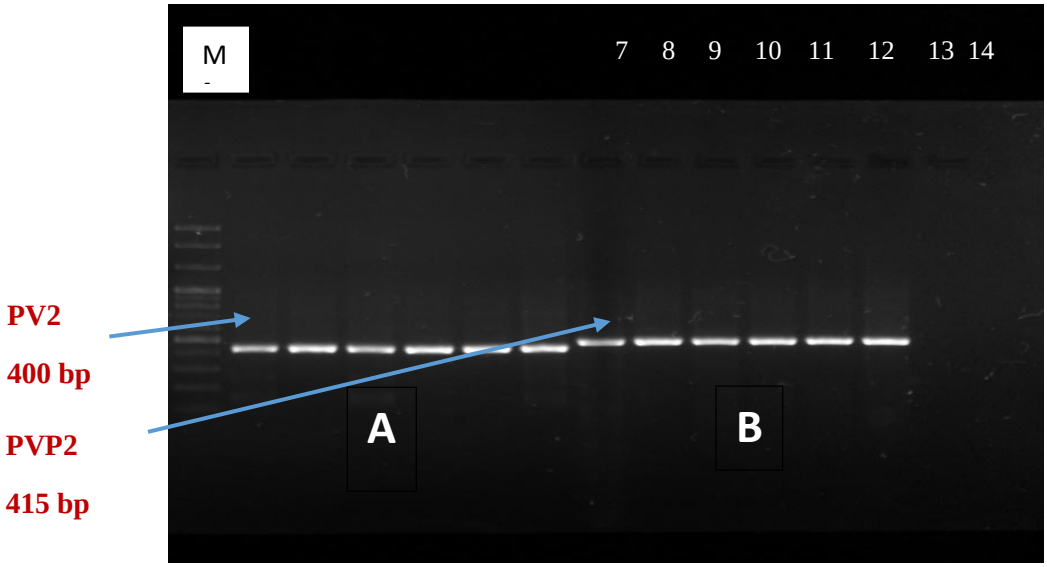

Fig. 7: Results of amplification (A: PV2, B: PVP2).

A:M Ladder (100 bp), 1-6 positive (400bp), 13 negative, 14 control negative.

B:M Ladder (100 bp), 7-12positive (420bp), 13 negative,14control negative.

Discussion:

Canine parvovirus is a serious famous virus in dogs worldwide, causing great danger to their health. The present study was carried out on 100 diseased dogs of different ages, breeds, and both sexes. (Ogbu et al.,2021;Behera et al.,2015)

The diseased dogs showed various clinical signs (Table 3), but the salient signs were fever (86%), loss of appetite (81%), and bloody diarrhea (62%), and the associated clinical signs were including the same three above signs together.

The infection rate of CPV was varied according to the animal sex, so the most clinical cases were appeared in males (64%) with infection rate up to (81.3%), while in females the collected clinical cases were slighter (36%) and the infection rate was also lower (66.7%). These results recorded from previous studies that refered to high percentage in male than female (Muzaffar et al., 2006;Castro et al., 2007;Islam et al.,2014;parthiban et al.,2015).while one study (Umar et al., 2015). refered to the opposite results(male41.5%, female58.5%). The infection rate differences in both sexes may related to physiological and hormonal effects (Jassim M.,2017).

Depending on the age, the dogs with age less than or up to 6 months were more susceptible to infection, so the dominant clinical cases were from these ages (62%) with infection rate up to

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(87.1%) whereas, in older doges the appeared cases (38%) and infection rate (57.9%) were least (Table 6). Many studies proved same results(Behera et al., 2015; Umar et al., 2015). . The susceptibility of these young ages to the infection may be attributed to the affinity of the CPV to proliferation in the mitotic cells, and these cells found in high rates in the intestine of the weaning ad young puppies(Islam et al., 2014).

The clinical cases appeared dominant in German shepherd (29%) dogs, while other breeds appeared less probable for infection and included Husky (14%), local breeds (12%), Doberman pinschers (11%), and terrier (10%) (Table 7).Some articles revealed same results that the high clinical cases were in forign breeds mainly German shepherd (Umar et al., 2015). These variations may be due to the sensitivity of the pure breeds to infection more than mixed breeds, so the inbreeding increase the resistancy of dogs to CPV, which maybe attributed to genetic factors (Castro *et al.*, 2007).

The distribution of the clinical cases according to the period of collection revealed that most of the cases appeared and were positive for CPV from November 2020 to January 2021 with the summit positivity and prevailing in December-January (Table 8). These results agreed with other previous studies (Jassim M.,2017;Dastmalchi Saei et al.,2016). that recorded the peak of infection was in December- January, While other researchers refered that the recorded cases were in spring to Autumn (Al-Bayati et al., 2010; Ling et al., 2012). The diversities in seasons of infection may be explained by variation in environmental conditions (Jassim M.,2017).

The results of the current study revealed that the detection of CPV by PCR technique (76%) was more accurate than rapid test (68%) (Table 9), nevertheless must taking into account that the difference between them is not very considerable. These results showed the reliability of the used tests and supporting the use of the rapid test as a presumptive diagnosis for CPV when the molecular techniques are missing. These results varied with other related studies, so one study (Jassim M.,2017). showed low accuracy of rapid test (25.4%) while another revealed a very near positivity rates (66%) to the current study when used rapid test(Al-Bayati et. al., 2010) . The variety of results may be due to the collection periods and the sheeding of virus, so the early collection of samples will give a n acurate results for rapid test (Jassim M.,2017). PCR was the principal technique for the detection of CPV and many researchers recommended to use it (Baba Sheikh et al.,2017; Dastmalchi Saei et al.,2016).because the viability of the virus is not necessary and the technique is highly specific and sensitive (Baba Sheikh et al.,2017; Dastmalchi Saei et al.,2016).

According to the results of positivity and negativity of PCR, both couples of primers (VP2, PVP2) showed similarity for the detection of CPV, which means the same numbers of positive and negative cases in both of them. So, each couple showed satisfactory output for detection of the DNA of CPV that was extracted in high purity and concentration(Buonavoglia et al., 2001;McEndaffer et al., 2017).

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In conclusion, the CPV is a principal disease in small and young household dogs in Mosul city and prevailing in the winter season. Both PCR technique and rapid test were efficient for diagnosis of CPV although the results of PCR were more accurate. Further study is needed for the diagnosis of the strains of CPV in household dogs in Mosul city.

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