PATHOLOGICAL AND MOLECULAR EVALUATION OF CANINE PARVOVIRUS IN SOUTH OF IRAQ

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ABSTRACT

This study is the first in southern Iraq, which shows the diagnosis of *Parvovirus* disease in young puppies, where the samples were collected from small dogs, and this was done from April 2018 to April 2019 it was conducted on 50 dogs with different breeds ranging between (2-18) months aged of both sexes. fecal samples were collected from infected dogs from veterinary clinics in (Basrah, Dhi Qar, Maysan and AL-Muthanna provinces) .After the autopsy of the dead carcass which died soon during the period (24-72) hours, bleeding in the intestines and congestion was observed as well as the water content and adhesions, hypertrophy of the heart and necrosis in areas of pale color of different size focci are surrounded by hyperemic zone. The microscopical result of enteric CPV lesion showed sever infiltration of inflammatory cells in the mucosal layer of intestine ,hypertrophy of goblet cells in the villi and necrotizing area in the tip of villi as well to hyperplasia of goblet cells also edematous fluid in mucosal layer. cardiac CPV lesion showed infiltration of inflammatory cells in myocardial cell fibers ,also there was an area of vacculation of some myocardial cells in addition to edematous fluid in the myocardial interstitiaum .significant increases of biochemical cardiac markers(cTi-1,AST,LDH and CPK) showed cardiac troponin I (cTnI), aspartate transaminase AST, creatinine phosphatkinase (CPK), and lactate dehydrogenase LDH which showed (0.024 ± 0.003),(50.5±4.49),(327.9 ± 55.01) and (467.9

±49.1) respectively when it is compared to healthy group which showed cardiac troponin I (cTnI), aspartate transaminase AST, creatinine phosphatkinase (CPK), and lactate dehydrogenase LDH were (0.004± 0.0003),(24.2±1.74),(90± 13.58) and (289 ±15.5), the Parvovirus contains 400 bp of matrimonial bases after being diagnosed using the polymerase chain reaction PCR and this technical method widely applied to provide rapid diagnosis The current study conducts that the CPV-2 is endemic in south of Iraq ,the disease shows very important clinical ,pathological and biochemical feature that lead to increase morbidity and mortality rate.

INTRODUCTION

Canine Parvovirus 2 (CPV2), a non-enveloped icosahedral virus with an approximate diameter of 20 nm, is a member of the parvovirus genus of the family, Parvovirus is a single stranded DNA virus with 5,200 nucleotides, the virus contains two structural (VP1 and VP2) and two non-structural proteins (NS1 and NS2) (11) also it is highly contagious and infectious agent characterized by hemorrhagic enteritis and myocarditis in neonatal puppies, and it is an important cause of death in young dogs (8). Parvovirus namely (CPV-2), the pathogen form and canine Parvovirus-1 (CPV-1) or the minute virus of canine (MVC), it was the first report of CPV enteritis in the United States of America (9), CPV-2 infection was reported in all sexes, ages and breeds of dogs (6; 10) it is Common causes of death in puppies that are less than 6 months of age (2, 18)month, Despite the aggressive treatment with treatments are available, many dogs still died because of CPV related Complications (13). *parvovirus* belongs to the genus Parvovirus of the family *Parvoviridae* (19).CPV-2 contains 3 antigen variants: Type 2a, 2b, and 2c. Which was first identified in the late 1970s, it was replaced by a few years after its emergence through antigen variants 2a and 2b, these two types are now distributed in worldwide (7). More recently, a third antigenic variant, CPV-2c has been

reported in Italy (5) .antigenic detection of CPV in Iraq occurred for the first time in 2010 (2). and its molecular detection took place in 2012 (1).

MATERIALS AND METHODS

Sample preperation

stool samples were collected from 50 dogs with different breeds ranging between (2-18) months aged from veterinary clinics and dog breeding in the period from April (2018) to April (2019), Conducted in four governorates in the provinces of Basrah, Dhi Qar, Maysan and Al-Muthana, all dogs suffered from clinical symptoms such as watery and bloody diarrhea and vomation, vaccination status, age and sex are reported All the samples were taken on ice so that the virus could last longer.

Pathological examination

Macroscopical feature after postmortem change at period (24-72) hours see the CPV enteric lesion and cardiac lesion, the Histological preparations of the internal organs (heart, intestine) were according to (3)

Clinical pathological parameter

Blood samples were collected from puppies from the cephalic vein in dog into tubes containing jell for evaluation of cardiac biomarkers for evaluation of serum cardiac biomarker were done using (COULTER COUNTER, RUBY Germany), Furthermore were also estimated according to (14).

Molecular detection

the molecular detection of parvovirus (PCR technique) by viral DNA extraction include the QIAamp® Fast DNA Stool Mini Kit according to manufacturer's instructions , Agarose gel electrophoresis is a method for determining the presence and size of PCR products PCR amplification of using VP2 gene specific primers (p1 and p2) was designed for specific-group detection of CPV-2

with expected size 400 bp (16,17) **Table .1** and reaction mixture PCR amplification was performed by using Accu power PCR premix (Bioneer Korea) at 20 µl **Table.2**

Table 1. Primers used in the study

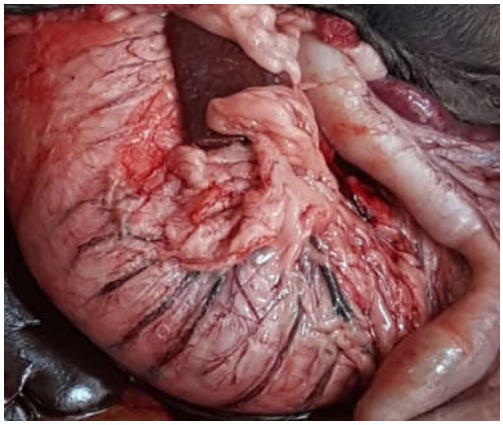
Primer	Sequence	Amplicon Size
CPV Primer 1 Forward	**CAAATAGAGCATTGGGCTTACC**	**400 bP**
CPV Primer 2 Reverse	**CAATCTCCTTCTGGATATCTTC**	

Table 2.Composition of reaction mixture (20µl) for PCR

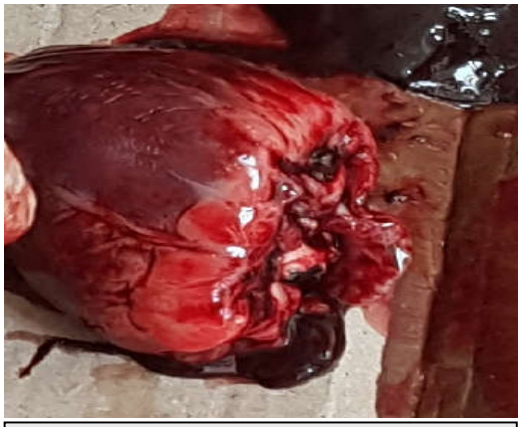
Components	Quantity (µl)
2X PCR Master Mix	5
Forward Primer (10 pmole/ µl)	1
Reverse Primer (10 Pmole/ µl	1
Nuclease free water	10
DNA template	3
total	20

RESULTS

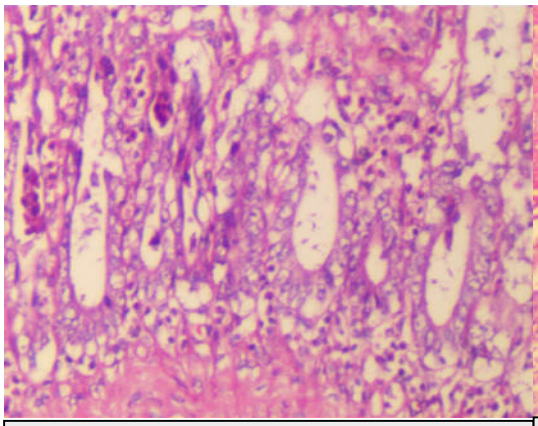
Macroscopical and microscopical results of enteric and cardiac CPV lesion showed sever hemorrhagic enteritis with congestion and enlargement of intestine as well to sloughing of intestinal mucosa and showed stages of myocarditis ,hypertrophy of heart as well to hyperemic zone of cardiac muscles (Fig1,2) also there was sever infiltration of inflammatory cells in the mucosal layer of intestine and myocardial cell fibers,hyperatrophy and hyperplasia of goblet cells in the villi and necrotizing area in the tip of villi edematous fluid in mucosal layer and myocardial interstitiaum ,also there was an area of vacculation of some myocardial cells and sever congestion myocardial blood vessels (Fig.3,.4)



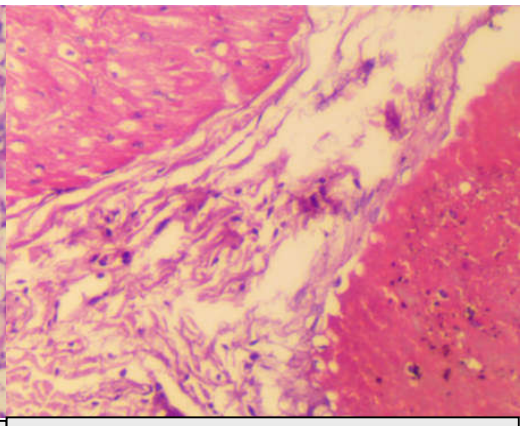
Figer1:small intestine showed hemorrhagic enteritis with enlargement



Figer 2: heart showed hypertrophy and myocarditis



Figer 3: showed sever infiltration inflammatory cells in the mucosal layer and hyperplasia in goblet cell of intestine

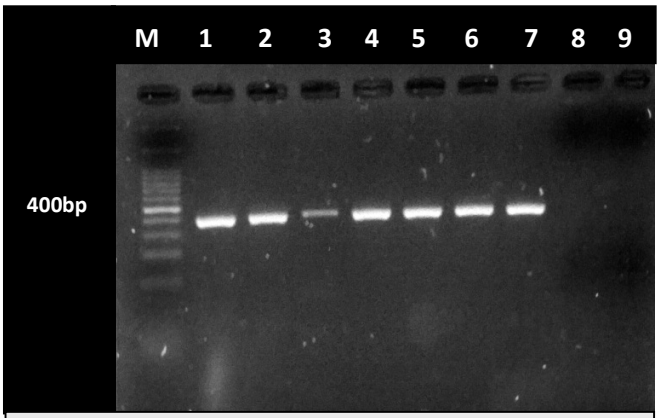


Figer 4: showed sever infiltration inflammatory cells in the myocardial fiber and edematous fluid in myocardial interstitiaum

The results of clinical pathology (biochemical test) showed significant ($p>0.05$) increase of cardiac troponin I (cTnI), aspartate transaminase AST, creatinine phosphatkinase (CPK), and lactate dehydrogenase LDH which showed (0.024 ± 0.003),(50.5±4.49),(327.9 ± 55.01) and (467.9 ±49.1) respectively when it is compared to healthy group which showed cardiac troponin I (cTnI), aspartate transaminase AST, creatinine phosphatkinase (CPK), and lactate dehydrogenase LDH were (0.004± 0.0003),(24.2±1.74),(90± 13.58) and (289 ±15.5) respectively (**table 3).**

Parameters	Healthy n=10	CPV n=50
cTnI ng/ml	0.004± 0.0003b	0.024 ± 0.003a
AST IU/L	24.2±1.74b	50.5±4.49a
CpK-MB IU/L	90± 13.58b	327.9 ± 55.01a
LDH IU/L	289 ±15.5b	467.9 ±49.1a

The result of molecular test CPV according to PCR amplification (400bp) showed the universal primer lane (M) is 100 bp DNA ladder marker, lane (1,2,3,4,5,6,) PCR Product ,lane (7) is the positive control and Lane (8,9) are negative (Fig 5)



Figer 5: pcr amplification

DISCUSSION

the current results of macroscopical and microscopical of enteric and cardiac CPV lesion showed sever hemorrhagic enteritis and myocarditis then sever infiltration of inflammatory cells in the mucosal layer of intestine and myocardial cell fibers, with vacculation of some myocardial cells and sever congestion myocardial blood vessels these results are in agreement with (12,18) whose reported the enteric and cardiac CPV lesion, and it is in agreement with

(4) who reported the second form of CPV is cardiac syndrome, or myocarditis, which can affect puppies ,The current biochemical result showed significant increase of Cardiac Troponin-I cTn-I aspartate transaminase (AST), creatinine phosphokinase (CPK), and lactate dehydrogenase (LDH) in infected puppies as compared to healthy group this result agreement with (15) who reported the troponins are released from damaged myocytes into the blood circulation and reach a measurable level within 4 hours and a peak level within 12-14 also the troponin levels may be rising because of the inability of the weak heart to maintain the proper coronary perfusion , the current of molecular result used set of primer (p1 and p2) and their reaction designed for detection about CPV-2 infection and its encode the viral protein (VP) (capsid protein) this result is in agreement with (16,17) that reported the primers of CPV-2 selected from different regions of the VP2 gene that encodes for the virus capsid protein the primers was designed for specific-group detection of CPV-2.

التقييم المرضي والجزيئي لمرض بارفو الكلاب في جنوب العراق

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الخلاصة

هذه الدراسة هي الأولى من نوعها في جنوب العراق ، والتي تُظهر تشخيص مرض بارفو فيروس في الجراء الصغار ، حيث تم جمع العينات من الكلاب الصغيرة ، للفترة بين أبريل ٢٠١٨ إلى أبريل ٢٠١٩ والتي أجريت على ٥٠ كلبًا بسلالات مختلفة تتراوح ما بين (٢-١٨) أشهر من كلا الجنسين ، تم جمع عينات برازيه من الكلاب المصابة من العيادات البيطرية في (محافظات البصرة وذي قار وميسان والمثنى). تشريح الجثة الميتة التي ماتت خلال فترة (٢٤-٧٢) ساعة لوحظ نزيف في الأمعاء والازدحام وكذلك محتوى الماء والالتصاقات وتضخم القلب ، نخر في مناطق ذات لون شاحب مختلفة الحجم وعقد محاط بالمنطقة الشديدة ، وأظهرت النتيجة المجهرية لآفة CPV المعوية تسللًا شديدًا للخلايا الالتهابية في الطبقة المخاطية للأمعاء ، وتضخم خلايا الكأس في منطقة الزغابة والنخر في طرف الزغب وكذلك لتضخم خلايا الكأس والسائل الوذمة في الطبقة المخاطية ، أظهرت آفة CPV القلبية تسلل الخلايا الالتهابية في أليف خلايا عضلة القلب ، وكذلك ظهور الفجوات في بعض خلايا عضلة القلب بالإضافة إلى السائل الوذمة في خلال عضلة القلب ، والزيادات الكبيرة في علامات القلب البيو كيميائية

(cTi-1 ، وأظهرت AST و LDH و CPK) تروبونين القلب (cTnI I) ، الأسبارتات ناقلة الأمين AST ، الكرياتينين فوسفاتكيناز (CPK) ، التي أظهرت (٠.٠٢٤ ± ٠.٠٠٣) ، (٥٠.٥ ± ٤.٤٩) ، (٣٢٧.٩ ± ٥٥.٠١) و (٤٦٧.٩ ± ٤٩.١) على التوالي عند مقارنتها بالمجموعة السليمة التي أظهرت تروبونين القلب (cTnI I) ، الأسبارتات الترانساميناز AST ، أسبرتات الترانساميناز AST ، الكرياتينين الفوسفاتينز (CPK) ، و LDH لاكتات الهيدروجين (٠.٠٠٤ ± ٠.٠٠٠٣) ، (٢٤.٢ ± ١.٧٤) ، (٩٠ ± ١٣.٥٨) و (٢٨٩ ± ١٥.٥) ،، يحتوي Parvovirus على ٤٠٠ نقطة أساس من القواعد الزوجية بعد تشخيصه باستخدام تفاعل البوليميرية سلسلة تفاعل PCR وهذه الطريقة التقنية تطبق على نطاق واسع لتوفير التشخيص السريع الدراسة الحالية التي أجريت أن CPV-2 متوطن في جنوب العراق ، أظهر المرض ميزة سريرية ومرضية وكيميائية حيوية مهمة للغاية تؤدي إلى زيادة معدل الإصابة بالأمراض والوفيات.

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