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ORIGINAL ARTICLE

ULTRASOUND FEATURES OF THE LIVER, SPLEEN, KIDNEY, AND HEART IN DOGS WITH CANINE PARVOVIRAL ENTERITIS

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ABSTRACT

The study was performed to determine the ultrasound features of the liver, spleen, kidney, and heart in dogs positive to canine parvoviral enteritis (CPE positive). Twenty-three CPE positive puppies and 14 apparently healthy puppies (CPE negative) with an age of less than one year and a body weight of less than 10 kg were utilized in the study. Two-dimensional ultrasonography was used to identify and evaluate the hepatic, renal, splenic, and echocardiographic structures while M-mode echocardiography was used to measure dimensions of various cardiac structures and calculate cardiac indices. No significant differences were found between the measurements of the CPE positive dogs and the CPE negative dogs. The results suggest that canine parvoviral enteritis does not produce any ultrasonographically visible abnormalities in the left kidney, liver, spleen, and heart of CPE positive dogs showing clinical signs such as bloody diarrhea and vomiting. Hence, ultrasonography of the aforementioned organs is of little value in the establishment of prognosis in dogs with active canine parvoviral enteritis.

Key words: *canine parvovirus, dog, echocardiography, enteritis, ultrasonography*

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INTRODUCTION

Canine parvovirus 2 (CPV-2) is a highly contagious, non-enveloped, single-stranded DNA virus known as the most common cause of canine parvoviral enteritis (CPE). CPE remains a significant worldwide disease due to its efficient feco-oral transmission, stability in the environment, and its ability to cause severe disease in dogs especially rapidly growing puppies between six weeks and six months of age. CPE has a 100% morbidity rate and frequent mortality rate of 91% in puppies and 10% in adult dogs (Appel *et al.*, 1978).

The establishment of a prognosis in cases of CPE is commonly based on complete blood count monitoring such that the presence of leukopenia is associated with poor prognosis (Castro, 2013). Although a complete blood count can give an insight on how well the body is fighting the virus, it cannot determine whether lesions have already developed in other organs of the body such as the liver, spleen, kidneys, and heart which may greatly affect the survival rate of the animal.

There have been very few major advancements regarding the treatment for CPE hence, the high fatality rate. When a dog is infected with the disease, treatment is limited to supportive and symptomatic. Moreover, treating a dog with CPE is financially and emotionally draining for pet owners and even for veterinarians (Acton and Ashton, 2013).

In response to this problem, this study was conducted to investigate the ultrasonographic features of the liver, spleen, and kidney and echocardiographic features in dogs with CPE to determine if the CPE virus has an effect on these organs aside from causing lesions in the intestines.

CPE is commonly encountered in the Philippines and the lack of proper treatment is a common occurrence in small animal practices due to financial constraints of the pet owner as the disease from a parvovirus infection is often costly. Nevertheless, even with treatment, the mortality rate is still quite high as there have been very few major advancements regarding the management of CPE.

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Previous studies have documented the presence of the virus in the heart, liver, kidney, spleen, thymus, lymph nodes, tonsils, and small intestines suggesting a broadened tissue tropism of CPV-2 (Nho *et al.*, 1997). CPV-2 infection in dogs can cause hepatic congestion and affects splenic nodules in the early course of the infection (Nandi and Kumar, 2010). Acute kidney injury in CPV-2 infected dogs can also be expected (van den Berg *et al.*, 2018). Myocarditis caused by CPV-2 infection can affect puppies in utero before eight weeks of age, and can cause a mortality of 70% in litters. It usually affects puppies of unvaccinated dams and since the percentage of dogs vaccinated against CPV-2 in the Philippines is low (Roble *et al.*, 2016), further studies on canine parvoviral myocarditis occurrence in the Philippines is needed.

The study was conducted to determine the ultrasound features of the heart, liver, kidney, and spleen in dogs positive for canine parvoviral enteritis as knowing the progression of the disease as it affects the heart, spleen, liver, and kidney will help clinicians to come up with a better prognosis and treatment plan, to gain a deeper insight into how the disease affects the body which will help in properly educating pet owners.

MATERIALS AND METHODS

An informed consent form was signed by the pet owner to facilitate inclusion of their pets for the study. The procedures performed on the patients were in accordance with the Department of Agriculture Administrative Order No. 40, Series of 1999, Section 1.3.2 stating that "diagnostic evaluation shall be exempted from the requirements of the IACUC protocol". Thirty-seven dogs of various breeds one year of age and below, weighing 1-9 kg were utilized in the study. These dogs were segregated into two groups; the first group consisted of 23 dogs that showed signs of acute watery to bloody diarrhea, vomiting, and lethargy and tested positive for canine parvovirus 2 (CPE-positive) using the SensPert® Canine Parvovirus Antigen Test Kit (VetAll Laboratories, Goyang-si, Gyeonggi-do, Korea) with 99.1% sensitivity and 100% specificity, while the second group consisted of 14 apparently healthy dogs (CPE-negative) that did not show any signs of vomiting, diarrhea, and lethargy. The CPE-positive dogs as well as the apparently healthy dogs were also grouped into large breed dogs and small breed dogs. Out of the 23 CPE-positive samples, five were classified as large breed dogs while 18 were classified as small breed dogs. For the CPE-negative dogs, nine out of 14 were classified as large breed dogs while five out of 14 were classified as small breed dogs. The samples were also grouped based on body weight. For the CPE-positive dogs, 14 out of 23 had a body weight range of 1.0–4.5 kg while nine out of 23 had a body weight range of 4.6–9 kg. For the CPE-negative dogs, on the other hand, eight out of 14 dogs had a body weight of 4.5–9.0 kg while six out of 14 dogs had a body weight of 1.0–4.5 kg.

An ultrasound machine (Eco 6 Vet Digital Color Doppler Ultrasound System, Chison Medical Technologies Co., Ltd., Wuxi, Jiangsu, China) equipped with a multi-frequency micro-convex scanner was used in the study. B-mode ultrasound features and echo mean values of the heart, liver, spleen, and left kidney as well as left renal dimensions, and echocardiographic measurements, and indices were compared across CPE negative and CPE positive dogs grouped according to breed size and body weight. The right kidney was not included in the study because of its location; it is extremely difficult to visualize the entire organ for mensuration.

Ultrasonography

B-mode ultrasonography of liver, spleen and kidneys

Liver

The dogs were placed on dorsal recumbency, and a microconvex scanner with a frequency of 6 MHz and a gain setting of 80 was placed below the xiphisternum. The different structures of the liver were visualized and the ultrasound features were noted. The echo mean values (EMV) of the liver parenchyma were determined using histogram analysis (luminosity) in Adobe Photoshop, Adobe Systems Inc., USA.

Spleen

The dogs were placed in a right lateral recumbency, and a micro convex transducer with a frequency of 6 MHz and a gain setting of 80 was placed in the left craniodorsal abdomen. The different structures of the spleen were visualized and the ultrasound features were noted. The echo mean values of the splenic parenchyma were determined using histogram analysis (luminosity) in Adobe Photoshop, Adobe systems Inc., USA.

Left Kidney

The patients were placed in a right lateral recumbency. The microconvex transducer with a frequency of 6 MHz and a gain setting of 80 was placed in the subcostal area, caudal to the last rib. The different structures of the left kidney were visualized and ultrasound features were noted.

The length of the left kidney was measured through a long axis view while the thickness and width were measured through a short axis view. Echo mean values of the renal medulla and renal cortex were determined using histogram analysis (luminosity) in Adobe Photoshop, Adobe Systems Inc., USA.

B-mode Echocardiography

The patients were positioned in both left and right lateral recumbency. The scanner was placed parasternally on the third to fourth intercostal spaces of both the left and the right side of the animal to examine the different structures of the heart including the aorta, atria, and the left and right ventricles.

M-mode echocardiography

Using M-mode, the right ventricular internal dimension at end-systole (RVIDs), end-diastole (RVIDd), right atrial (RA) diameter, diastolic and systolic left ventricular chamber measurements (LVIDd and LVIDs), right ventricular wall diastolic thickness (RVWd) and systolic thickness (RVWs), left ventricular free wall diastolic thickness (LVWd) and systolic thickness (LVWs), left atrial diameter (LA), aortic root dimension (Ao), and left atrial to aortic root ratio (LA/Ao) were measured.

Echocardiographic indices

The fractional shortening (FS), the left ventricle ejection fraction (EF), the left diastolic volume (LVVd), the left ventricle stroke volume (SV), and the left ventricle systolic volume (LVVs) were calculated based on the M-mode measurements using Teicholz equation reported by Boon (2011).

Data Analysis

Mean±SD of the measurements for dimensions and echo mean values were computed. The data were first tested for normality using the Kolmogorov-Smirnov test for normality. A two-tailed independent Student t-test with a significance level of 0.05 was used to determine if there are any significant differences among the data sets between CPE-negative and CPE-positive dogs.

RESULTS AND DISCUSSION

Liver

For both CPE-positive and CPE-negative dogs, the parenchyma of the liver appeared to be homogenous and hypoechoic and the gallbladder appeared as a round to pear-shaped structure with thin hypoechoic walls and an anechoic lumen (Figures 1a and 1b). This is consistent with the normal ultrasonographic features of the canine liver as reported by Mattoon and Nyland (1995), Farrow (2003), Mannion (2006), and Dennis (2010). In both groups, the portal vein appeared as an anechoic lumen with a hyperechoic wall while the hepatic veins appeared as anechoic channels with hypoechoic walls. These results cohere with a study on the echogenicity of portal and hepatic veins of healthy dogs by Wachsberg *et al.* (1998). Upon comparison of the EMVs of the hepatic parenchyma of CPE-positive and CPE-negative dogs based on breed size and body weight, no significant differences were noted (Tables 1 and 2).

According to a study by Nandi and Kumar (2010), pulmonary edema and passive congestion of the liver with variable degrees of ascites and pleural effusion are commonly observed in patients with CPV-2 myocarditis. Moreover, Carpenter *et al.* (1980) documented the presence of hepatomegaly and blood-tinged ascitic fluid in puppies dying of CPV-2 myocarditis. Hepatic congestion, which is commonly caused by right-sided cardiac insufficiency leading to increased pressure within the caudal vena cava and hepatic veins, is visualized via ultrasonography as dilated CVC, dilated hepatic veins, and an enlarged (hepatomegaly) and diffusely hypoechoic liver. None of the samples in this study showed dilated veins and diffusely hypoechoic liver.

Further studies are warranted to confirm whether CPV-2 myocarditis that causes liver lesions may coexist with CPE. Nandi and Manoj (2010) stated that diarrhea is not seen in the cardiac form of CPV-2 since the site of viral multiplication is the muscle cells of the immature heart, while Kittleson and Kienle (1998) declared that puppies infected with CPE may show both myocarditis and acute diarrhea. The absence of liver lesions associated with cardiac insufficiency in all the CPE-positive dogs in this study may suggest that the presence of the CPV-2 myocarditis is unlikely. Nho *et al.* (1997) detected the presence of the CPV-2 virus in liver sections via in-situ hybridization, however, since no notable changes were seen in the ultrasound features of the liver of the CPE-positive dogs, it can be said that the virus does not cause hepatic lesions visible through B-mode ultrasonography.

Spleen

The spleen of both CPE-negative and CPE-positive dogs appeared as a homogenously fine-textured tongue-shaped structure relatively hyperechoic to the liver and renal cortices and

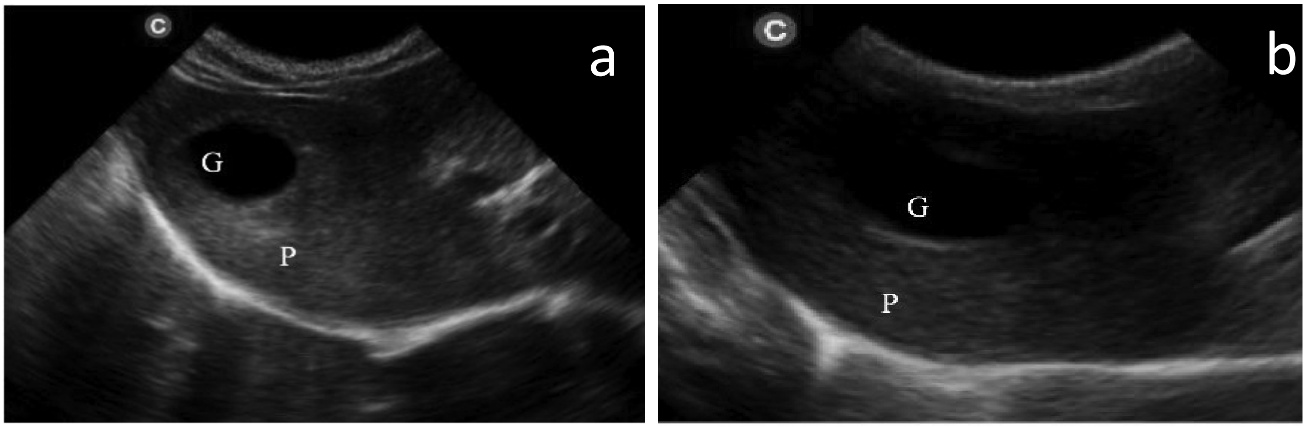

Figure 1. Short axis view of the hepatic parenchyma (P) and gallbladder (G) of a canine parvoviral enteritis- positive dog (a) and a canine parvoviral enteritis- negative dog (b).

Table 1. Echo mean values (mean±SD) of the parenchyma of the liver, spleen and kidneys in canine parvoviral enteritis (CPE)-positive dogs and CPE-negative dogs based on body weight.

Echo mean values	CPE-negative dogs		CPE-positive dogs	
Hepatic parenchyma				
1.0 – 4.5 kg	n=6	46.92±23.56	n=12	33.67±14.19
4.5 – 9.0 kg	n=8	35.02±16.97	n=9	46.69±29.93
Total	n=14	40.12±20.15	n=21	37.55±22.69
Splenic parenchyma				
1.0 – 4.5 kg	n=6	27.93±15.42	n=11	29.03±25.68
4.5 – 9.0 kg	n=8	27.76±14.60	n=9	23.71±10.92
Total	n=14	17.38±14.77	n=20	25.13±21.48
Renal cortex				
1.0 – 4.5 kg	n=6	42.4±14.30	n=9	27.15±18.78
4.5 – 9.0 kg	n=9	29.69±14.82	n=4	25.16±8.81
Total	n=15	34.04±15.81	n=13	25.43±15.01
Renal medulla				
1.0 – 4.5 kg	n=6	10.99±6.35	n=9	16.19±16.33
4.5 – 9.0 kg	n=8	2.62±2.51	n=9	6.57±7.15
Total	n=14	6.21±6.12	n=18	11.37±13.20

Table 2. Echo mean values (mean±SD) of the parenchyma of the liver, spleen and kidneys in canine parvoviral enteritis (CPE)-positive dogs and CPE-negative dogs based on breed size.

Echo mean values	CPE-negative dogs		CPE-positive dogs	
Hepatic parenchyma				
Small breed	n=5	39.78±17.65	n=16	36.09±16.72
Large breed	n=9	40.31±22.44	n=5	46.83±37.52
Total	n=14	40.12±20.15	n=21	37.55±22.69
Splenic parenchyma				
Small breed	n=5	27.24±17.13	n=15	27.88±22.66
Large breed	n=9	12.46±10.96	n=5	16.89±16.78
Total	n=14	17.38±14.77	n=20	25.13±21.48
Renal cortex				
Small breed	n=5	41.86±15.91	n=14	25.51±16.63
Large breed	n=9	29.69±14.82	n=4	25.16±8.81
Total	n=14	34.04±15.81	n=18	25.43±15.01
Renal medulla				
Small breed	n=5	12.81±5.05	n=14	12.43±14.50
Large breed	n=9	2.54±2.37	n=4	7.69±7.27
Total	n=14	6.21±6.12	n=18	11.37±13.20

surrounded by a thin hyperechoic capsule (Figures 2a and 2b). The branches of the splenic veins of both groups appeared as tubular anechoic structures. These findings are consistent with the normal description of the canine spleen as established by Nyland *et. al* (1995) and Mareschal *et al.* (2007). No significant differences were noted in the ultrasound features and EMVs of the CPE-positive and the CPE-negative dogs grouped based on breed size and body weight (Tables 1 and 2). Measurement of the splenic size was not performed since according to Hanson *et al.* (2001), ultrasonographic assessment of splenic size is subjective and can vary greatly particularly among several breeds. For instance, the spleen of German Shepherd dogs and Greyhounds are known to be bigger compared to that of other breeds (Mahoney, 2012).

In the pathogenesis of CPV infection, after ingestion of the virus and initial replication in the lymphatic tissues of the oropharynx, the virus begins to replicate in the thymus, spleen, and bone marrow one to three days after infection. (Pollock, 1982) Moreover, studies by Lin and Chiang (2016) and Nho *et. al* (1997) have documented the presence of CPV-2+ in splenic tissues however, the results suggest that it does not cause ultrasonographically visible lesions on the splenic tissues of CPE positive dogs with acute diarrhea, vomiting, and lethargy.

Left kidney

In this study, only the left kidney was observed and measured since it is easy to visualize through a subcostal ventrolateral approach, unlike the right kidney which is difficult to visualize due to its location in the cranial abdomen and because of the gas bowel that may obstruct the full view of the organ. A lateral approach through the 11th to 12th intercostal space is thought to be necessary for better visualization (D'Anjou *et al.*, 2015). However, since this study utilizes puppies, the intercostal space is too narrow to fit the entire micro-convex scanner leading to obscuration of the view of the kidney by the ribs.

The left kidney of both groups appeared as a bean-shaped structure with a hypoechoic renal cortex, an anechoic renal medulla separated into lobules by interlobar vessels, and a hyperechoic renal sinus surrounding the renal pelvis (Figures 3a and 3b). These features are consistent with that of the normal renal ultrasonographic features as described by D'Anjou (2015).

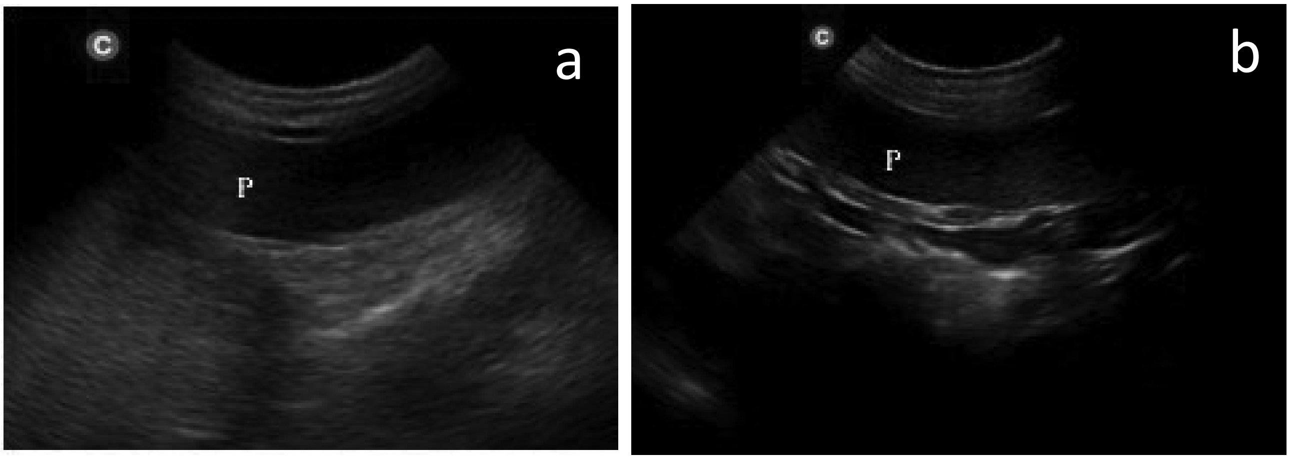

Figure 2. Long axis view of the splenic parenchyma (P) of a canine parvoviral enteritis-positive dog (a) and canine parvoviral enteritis-negative dog (b).

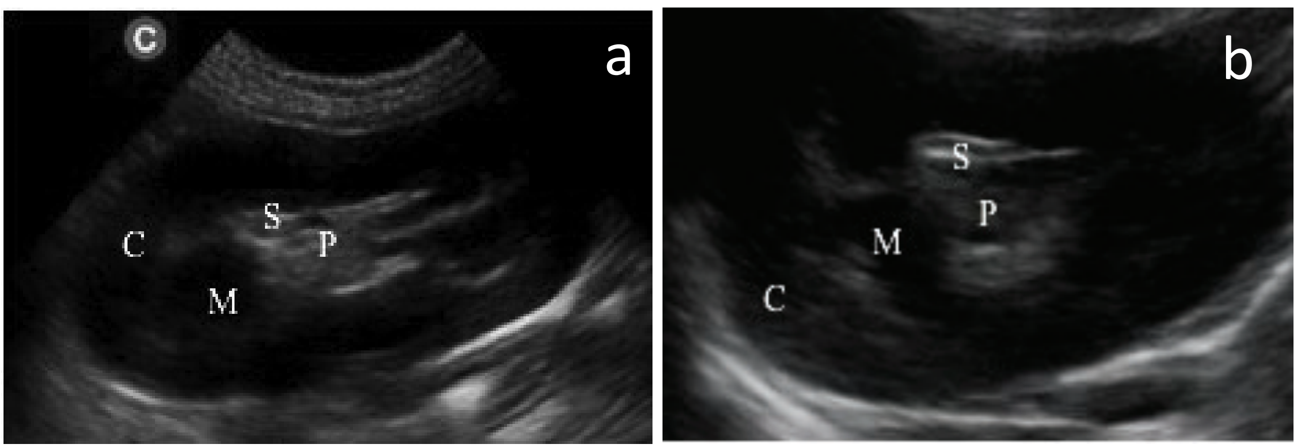

Figure 3. Long axis view of the left kidney of a canine parvoviral enteritis-positive dog (a) and canine parvoviral enteritis-negative dog (b) showing the medulla (M), cortex (C), renal pelvis (P), and renal sinus (S)

The EMVs of the cortex and medulla of all the CPE-positive and CPE-negative groups showed no significant differences (Tables 1 and 2). For the dimensions of the left kidney, the length, width, thickness, and area, none of the measurements showed a significant difference among all groups of CPE-positive and CPE-negative dogs (Tables 3 and 4). Furthermore, the left renal dimensions of CPE-positive and CPE-negative dog groups based on body weight and breed size showed that large breed dogs had higher left renal dimensions than small breed dogs, and dogs weighing 4.6-9kg had higher left renal dimensions than dogs weighing 1-4.5kg. This means that the left renal dimensions are correlated with breed size as stated in a study by Barr *et al* (1990).

Van den Berg *et al.* (2018) reported that dogs infected with canine parvovirus are at risk of developing acute kidney injury due to various factors such as severe dehydration, hypotension, and sepsis. Ultrasound features of acute kidney injury include enlargement and hypoechogenicity, attenuation of the corticomedullary distinction, dilation of renal pelvis and diverticuli, and dilation of the proximal ureter with highly echogenic urine (Mattoon and Nyland, 2015). These signs were not observed in the samples obtained hence, acute kidney injury may be ruled out.

Table 3. Dimensions of the left kidney in canine canine parvoviral enteritis (CPE)-positive dogs and CPE-negative dogs based on body weight.

Parameters	CPE-negative dogs		CPE-positive dogs	
Length (cm)				
1.0 – 4.5 kg	n=6	3.65±0.32	n=9	3.40±0.39
4.5 – 9.0 kg	n=7	4.51±0.47	n=8	4.12±0.74
Total	n=13	4.11±0.59	n=17	3.74±0.67
Width (cm)				
1.0 – 4.5 kg	n=5	2.47±0.38	n=7	2.22±0.20
4.5 – 9.0 kg	n=7	2.72±0.35	n=8	2.67±0.11
Total	n=12	2.62±0.37	n=15	2.46±0.28
Thickness (cm)				
1.0 – 4.5 kg	n=6	2.02±0.12	n=9	1.76±0.34
4.5 – 9.0 kg	n=8	2.24±0.06	n=9	2.20±0.33
Total	n=14	2.15±0.05	n=18	1.98±0.39
Area (cm^2)				
1.0 – 4.5 kg	n=6	23.45±2.86	n=9	18.93±5.16
4.5 – 9.0 kg	n=7	35.01±5.99	n=8	31.02±7.47
Total	n=13	29.68±7.57	n=17	24.62±8.74

Table 4. Dimensions of the left kidney in canine parvoviral enteritis (PCE)-positive dogs and CPE-negative dogs based on breed size.

Parameters	CPE-negative dogs		CPE-positive	
Length (cm)				
Small breed	n= 5	3.59±0.32	n=13	3.71±0.64
Large breed	n= 8	4.44±0.48	n=4	3.83±0.89
Total	n=13	4.11±0.59	n=17	3.74±0.67
Width (cm)				
Small breed	n=4	2.43± 0.43	n=12	2.40±0.28
Large breed	n=8	2.71±0.33	n=3	2.71±0.09
Total	n=12	2.62±0.37	n=15	2.46±0.28
Thickness (cm)				
Small breed	n=5	2.05±0.11	n=14	1.94±0.40
Large breed	n=9	2.21±0.26	n=4	2.14±0.38
Total	n=14	2.15±0.05	n=18	1.98±0.39
Area (cm^2)				
Small breed	n=5	23.02±2.97	n=13	22.66±7.74
Large breed	n=8	33.84±6.46	n=4	30.99±9.19
Total	n=13	29.68±7.57	n=17	24.62±8.74

Heart

In this study, the patients were placed in lateral recumbency for both left and right parasternal approaches. A frequency of 6 MHz was used, complying to the standard guidelines of using a 4-8 MHz transducer frequency for dogs weighing 5-40 kilograms. (D' Anjou *et al.*, 2015).

Upon B-mode ultrasonography of both CPE-positive and CPE-negative dogs, the walls of the left ventricle, right ventricle and interventricular septum appeared hypoechoic, while the chambers of the left and right ventricles appeared anechoic (Figures 4a and 4b). No bowing was observed in the interventricular septum, ruling out the presence of volume overload in the ventricles (Boon, 2011). The aorta of both groups appeared as a circular anechoic structure with hyperechoic walls while the atria appeared as anechoic structures near the aorta (Figures 5a and 5b). This description is consistent with that of a normal canine heart as reported by Boon (2011).

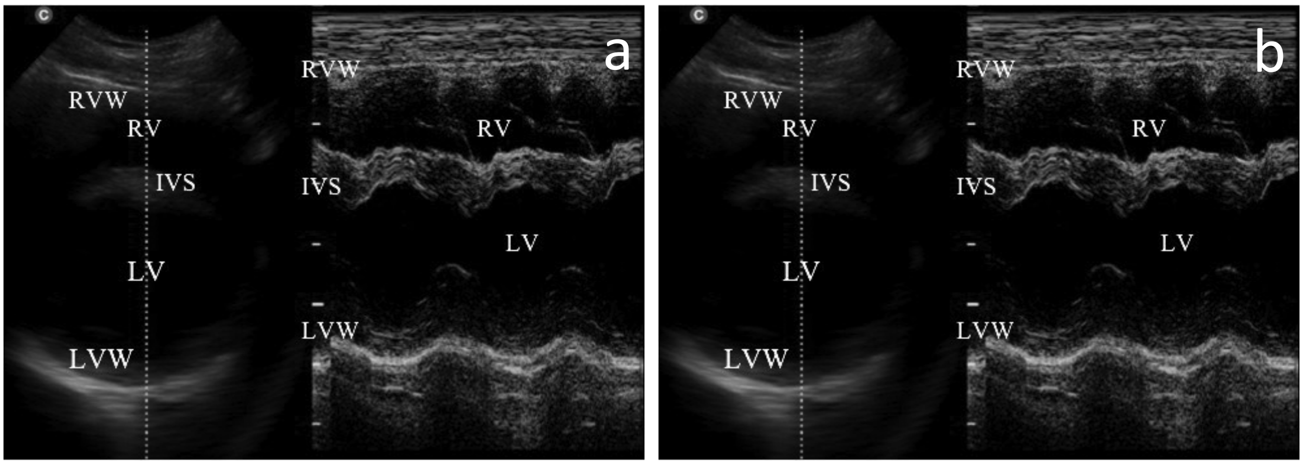

Figure 4. Right parasternal short axis view of the heart of a canine parvoviral enteritis-positive dog (a) and canine parvoviral enteritis-negative dog (b), showing the right ventricular wall (RVW), right ventricle (RV), interventricular septum (IVS), left ventricle (LV), and left ventricular wall (LVW).

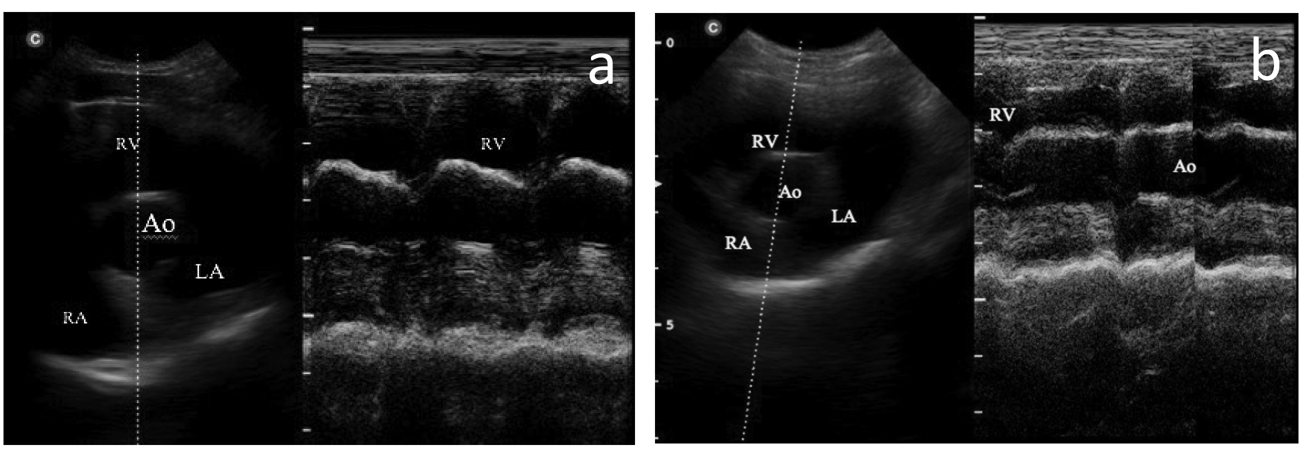

Figure 5. Left parasternal short axis view of the heart of a canine parvoviral enteritis-positive dog (a) and canine parvoviral enteritis-negative dog (b), showing the aorta (Ao), right atrium (RA), left atrium (LA), and right ventricle (RV).

For the echocardiographic measurements of the left ventricle, no significant differences were observed between the LVWs, LVWd, LVIDd, and LVIDs of the CPE-positive and CPE-negative dogs when grouped according to breed size and body weight (Tables 5 and 6).

For the right ventricle, no significant differences were seen between the RVIDd, RVIDs, RVWd, and RVWs of the CPE-positive dogs and CPE-negative dogs across all groups (Table 5a). The RVWd values of all the CPE-positive and CPE-negative dogs were normal since these values are approximately ½ of the LVWd values (Boon, 2011). Furthermore, in both CPE-positive and CPE-negative dogs, the values of the LVWs and LVWd appeared higher than the RVWs and RVWd, respectively (Tables 5a and 5b). This is consistent with the normal anatomy of the canine heart where the walls of the left ventricle are known to be 1.6 to 3 times thicker than the right ventricle (Queiroz *et al.*, 2018).

Table 5a. Echocardiographic measurements (mean±SD) in canine parvoviral enteritis (CPE)-positive dogs and CPE-negative dogs based on breed size.

Parameters	CPE-negative dogs		CPE-positive dogs	
RVIDd (cm)				
Small breed	n=3	0.88±0.17	n=15	0.77±0.21
Large breed	n=4	1.00±0.11	n=5	0.99±0.22
Total	n=7	0.95±0.14	n=20	0.82±0.24
RVIDs (cm)				
Small breed	n=3	0.49±0.06	n=14	0.47±0.17
Large breed	n=3	0.60±0.04	n=5	0.54±0.10
Total	n=6	0.55±0.08	n=19	0.49±0.16
LVIDd (cm)				
Small breed	n=5	1.51±0.31	n=15	1.50±0.42
Large breed	n=5	1.79±0.28	n=5	1.68±0.58
Total	n=10	1.65±0.31	n=20	1.55±0.46
LVIDs (cm)				
Small breed	n=5	0.84±0.31	n=14	0.93±0.33
Large breed	n=5	1.05±0.29	n=5	1.02±0.49
Total	n=10	0.95±0.31	n=19	0.95±0.36
RVWs (cm)				
Small breed	n=3	0.77±0.14	n=12	0.71±0.13
Large breed	n=4	0.81±0.08	n=5	0.65±0.14
Total	n=7	0.80±0.10	n=17	0.69±0.12

RVIDd: right ventricular internal diameter at diastole, RVIDs: right ventricular internal diameter at systole, LVIDd: left ventricular internal diameter at diastole, LVIDs: left ventricular internal diameter at systole, RVWs: right ventricular wall at systole

Table 5b. Echocardiographic measurements (mean±SD) in canine parvoviral enteritis (CPE)-positive dogs and CPE-negative dogs based on breed size.

Parameters	CPE-negative dog		CPE-positive dogs	
LVWs (cm)				
Small breed	n=5	1.00±0.21	n=16	0.98±0.21
Large breed	n=5	1.04±0.17	n=5	0.94±0.18
Total	n=10	1.02±0.18	n=21	0.91±0.20
LVWd (cm)				
Small breed	n=5	0.64±0.09	n=14	0.59±0.21
Large breed	n=5	0.64±0.10	n=5	0.63±0.16
Total	n=10	0.64±0.09	n=19	0.59±0.21
RVWd (cm)				
Small breed	n=3	0.43±0.06	n=10	0.36±0.04
Large breed	n=4	0.37±.0.05	n=4	0.28±0.08
Total	n=7	0.39±0.05	n=14	0.37±0.04
RA (cm)				
Small breed	n=3	0.88±0.08	n=10	0.79±0.09
Large breed	n=5	0.78±0.18	n=2	0.78±0.27
Total	n=8	0.82±0.15	n=12	0.79±0.12
LA (cm)				
Small breed	n=2	0.98±0.05	n=12	0.98±0.14
Large breed	n=3	1.14±0.05	n=4	1.17±0.02
Total	n=5	1.08±0.10	n=16	1.03±0.15
Ao (cm)				
Small breed	n=5	1.03±0.16	n=14	1.01±0.18
Large breed	n=5	1.30±0.14	n=5	1.14±0.14
Total	n=10	1.16±0.20	n=19	1.04±0.18

LVWs: left ventricular wall at systole, LVWd: left ventricular wall at diastole, RVWd: right ventricular wall at diastole, RA: right atrium, LA: left atrium, Ao: aortic root.

Table 6a. Echocardiographic measurements (mean±SD) in canine parvoviral enteritis (CPE)-positive dogs and CPE-negative dogs based on body weight.

Parameters	CPE-negative dogs		CPE-positive dogs	
RVIDd (cm)				
1.0 – 4.5 kg	n=3	0.88±0.17	n=11	0.73±0.21
4.5 – 9.0 kg	n=4	1.00±0.11	n=9	0.95±0.19
Total	n=7	0.95±0.14	n=20	0.82±0.24
RVIDs (cm)				
1.0 – 4.5 kg	n=3	0.49±0.06	n=10	0.43±0.18
4.5 – 9.0 kg	n=3	0.60±0.04	n=9	0.54±0.12
Total	n=6	0.55±0.08	n=19	0.49±0.16
LVIDd (cm)				
1.0 – 4.5 kg	n=6	1.48±0.29	n=11	1.43±0.41
4.5 – 9.0 kg	n=4	1.90±0.12	n=9	1.69±0.50
Total	n=10	1.65±0.31	n=20	1.55±0.46
LVIDs (cm)				
1.0 – 4.5 kg	n=6	0.82±0.29	n=10	0.87±0.31
4.5 – 9.0 kg	n=4	1.14±0.26	n=9	1.05±0.42
Total	n=10	0.95±0.31	n=19	0.95±0.36
RVWs (cm)				
1.0 – 4.5 kg	n=3	0.73±0.14	n=9	0.69±0.13
4.5 – 9.0 kg	n=4	0.81±0.08	n=8	0.70±0.11
Total	n=7	0.80±0.10	n=17	0.69±0.12

RVIDd: right ventricular internal diameter at diastole, RVIDs: right ventricular internal diameter at systole, LVIDd: left ventricular internal diameter at diastole, LVIDs: left ventricular internal diameter at systole, RVWs: right ventricular wall at systole

Table 6b. Echocardiographic measurements (mean±SD) in canine canine parvoviral enteritis (CPE)-positive dogs and CPE-negative dogs based on body weight

Parameters	CPE-negative dogs		CPE-positive dogs	
LVWs (cm)				
1.0 – 4.5 kg	n=6	1.00±0.19	n=12	0.99±0.21
4.5 – 9.0 kg	n=4	1.05±0.19	n=9	0.96±0.21
Total	n=10	1.02±0.18	n=21	0.91±0.20
LVWd (cm)				
1.0 – 4.5 kg	n=5	0.66±0.10	n=10	0.60±0.15
4.5 – 9.0 kg	n=4	0.61±0.09	n=9	0.63±0.23
Total	n=9	0.64±0.09	n=19	0.59±0.21
RVWd (cm)				
1.0 – 4.5 kg	n=3	0.43±0.06	n=8	0.31±0.06
4.5 – 9.0 kg	n=4	0.42±0.04	n=8	0.29±0.10
Total	n=7	0.42±0.04	n=16	0.30±0.08
RA (cm)				
1.0 – 4.5 kg	n=2	0.67±0.26	n=8	0.78±0.10
4.5 – 9.0 kg	n=4	0.85±0.09	n=4	0.79±0.16
Total	n=6	0.79±0.16	n=12	0.79±0.12
LA (cm)				
1.0 – 4.5 kg	n=2	0.98±0.05	n=10	0.95±0.11
4.5 – 9.0 kg	n=3	1.14±0.05	n=6	1.15±0.11
Total	n=5	1.08±0.10	n=16	1.03±0.15
Ao (cm)				
1.0 – 4.5 kg	n=5	1.03±0.16	n=11	0.96±0.14
4.5 – 9.0 kg	n=5	1.30±0.14	n=8	1.14±0.16
Total	n=10	1.16±0.20	n=19	1.04±0.18

LVWs: left ventricular wall at systole, LVWd: left ventricular wall at diastole, RVWd: right ventricular wall at diastole, RA: right atrium, LA: left atrium, Ao: aortic root

For the aorta and the left and right atria, no significant differences were observed between the LA, RA, Ao, and LA/Ao of the CPE-positive and CPE-negative dogs grouped according to breed size and body weight. According to Franco *et al*. (2016), body weight and body surface area (BSA) are positively correlated with aortic and left atrial dimensions. Assessment of these dimensions may be done using the aortic root size and its ratio to the left atrium. LA to AOD ratio is commonly used to characterize atrial dilation and is also known to provide a reliable value as the relationship of the aorta to the chambers is fixed (Minors *et* al.,2006). According to Boon (2011), the normal established LA/Ao range is 0.83-1.13 regardless of body surface area and weight. However, according to Wesselowski *et al*. (2014), LA/Ao is deemed normal if it is less than or equal to 1.5. The LA/Ao values of the CPE-positive samples fall between both normal ranges (Tables 7 and 8). For the right atrium, there are currently no established reference ranges for the canine species.

Various researches have shown that a cardiac form of CPV-2 has been known to affect puppies in the form of myocardial fibrosis. In the study by Hayes *et al*. (1979), gross lesions of the heart seen in parvoviral myocarditis may include cardiac enlargement with dilated left atrium and ventricle. Moreover, survivors of neonatal infection may show scarring and thinning of the myocardium (Kittleson and Kienle, 1998).

However, as mentioned previously, more studies are warranted to confirm whether the cardiac form of CPV-2 may co-occur with CPE. Nandi and Manoj (2010) stated that diarrhea and enteritis cannot be observed in the cardiac form of CPV-2 because the cardiac form of the virus only replicates in the muscle cells of the immature heart and not in the crypts of Lieberkuhn which is the case for CPE. Conversely, Lenghaus *et al.* (1980) have documented several cases in which dogs dying of CPE had parvovirus intranuclear inclusion bodies in myocardial cells despite having no obvious signs of myocarditis. Moreover, Ford *et al.* (2017) also identified myocardial CPV-2 in juveniles with minimal myocarditis and CPV-2 enteritis.

Table 7. Echocardiographic indices (mean±SD) in canine parvoviral enteritis (CPE)-positive dogs and CPE-negative dogs based on body weight

Indices	CPE-negative dogs		CPE-positive dogs	
FS				
1.0 – 4.5 kg	n=6	44.16±15.67	n=10	40.46±17.92
4.5 – 9.0 kg	n=4	40.30±12.76	n=9	38.94±12.65
Total	n=10	42.61±13.96	n=19	39.74±15.24
EF				
1.0 – 4.5 kg	n=6	80.12±11.81	n=10	70.53±21.63
4.5 – 9.0 kg	n=4	73.06±14.32	n=9	70.62±16.03
Total	n=10	77.29±12.61	n=19	70.57±18.66
LA/Ao				
1.0 – 4.5 kg	n=2	0.91±0.05	n=10	0.95±0.11
4.5 – 9.0 kg	n=3	1.14±0.05	n=6	1.16±0.11
Total	n=5	0.91±0.06	n=16	0.96±0.07
SV				
1.0 – 4.5 kg	n=6	5.61±2.56	n=11	4.53±3.47
4.5 – 9.0 kg	n=4	8.64±2.39	n=9	6.37±4.57
Total	n=10	6.82±2.82	n=20	5.35±4.00
LVVd				
1.0 – 4.5 kg	n=6	7.09±3.30	n=11	6.15±3.81
4.5 – 9.0 kg	n=4	11.79±2.41	n=9	9.45±6.23
Total	n=10	8.97±3.72	n=20	7.63±5.17
LVVs				
1.0 – 4.5 kg	n=6	1.49±1.57	n=10	1.78±1.69
4.5 – 9.0 kg	n=4	3.15±1.74	n=9	3.08±2.68
Total	n=10	2.15±1.77	n=19	2.39±1.77

FS: fractional shortening, EF: ejection fraction, LA/Ao: left atrium to aortic root ratio, SV: stroke volume, LVVd: left ventricular diastolic volume, LVVs: left ventricular systolic volume

Table 8. Echocardiographic indices (mean±SD) in canine parvoviral enteritis (CPE)-positive dogs and CPE-negative dogs based on breed size.

Indices	CPE-negative dogs		CPE-positive dogs	
FS				
Small breed	n=5	43.97±17.52	n=14	39.47±15.35
Large breed	n=5	41.26±11.26	n=5	40.49±16.69
Total	n=10	42.62±13.96	n=19	39.74±15.24
EF				
Small breed	n=5	79.95±13.19	n=14	70.36±18.36
Large breed	n=5	74.64±12.90	n=5	71.17±21.68
Total	n=10	77.29±12.61	n=19	70.57±18.66
LA/Ao				
Small breed	n=2	0.82±0.08	n=12	0.95±0.27
Large breed	n=3	0.90±12.90	n=4	0.99±21.68
Total	n=5	0.91±0.06	n=16	0.96±0.07
SV				
Small breed	n=5	6.00±2.65	n=15	4.95±3.33
Large breed	n=5	7.64±3.05	n=5	6.59±5.90
Total	n=10	6.82±2.82	n=20	5.35±4.00
LVVd				
Small breed	n=5	7.62±3.40	n=15	6.95±4.39
Large breed	n=5	10.33±3.88	n=5	9.68±7.28
Total	n=10	8.97±3.72	n=20	7.63±5.17
LVVs				
Small breed	n=5	1.61±1.71	n=14	2.14±1.99
Large breed	n=5	2.69 ±1.82	n=5	3.10±3.02
Total	n=10	2.15±1.77	n=19	2.39±1.77

FS: fractional shortening, EF: ejection fraction, LA/Ao: left atrium to aortic root ratio, SV: stroke volume, LVVd: left ventricular diastolic volume, LVVs: left ventricular systolic volume.

According to Jeserich *et al.* (2009), myocarditis can only be definitively diagnosed via histological, immunohistological, and molecular techniques. They also said that the presence of myocardial interstitial edema in acute myocarditis leads to thickening of the ventricular wall, which can be detected by echocardiography.

In this present study, thickening of the ventricular wall as well as cardiac enlargement with dilated left atrium and ventricle were not observed. Thus, the presence of the cardiac form of CPV-2 coexisting with CPE is unlikely.

The lack of significant differences among all the ultrasound features of the different organs across various groups may be because ultrasonographic examination was done during the acute stage of the disease where distinct clinical signs are exhibited which is known to occur three to seven days after infection (Nandi and Manoj, 2010). Because of the fast progression of the disease, there may be little time for the virus to cause ultrasonographically visible changes in the organs examined. Based on the above results, it can be concluded that CPV-2 does not produce any ultrasonographically visible abnormalities in the left kidney, liver, spleen, and heart of CPE positive dogs showing clinical signs such as bloody diarrhea and vomiting. Therefore, ultrasonography of the left kidney, liver, spleen, and heart is of little value in the establishment of prognosis in dogs with active canine parvoviral enteritis.

Some of the organ dimensions were not measured because accurate visualization of the structures could not be conducted. These could be attributed to the difficulty in the restraint and positioning of some animals which were relatively intractable and the presence of a thick layer of subcutaneous fat.

Further studies to determine the relationship between the ultrasound features of the heart, liver, kidney, and spleen in dogs with other infectious diseases such as *Ehrlichiosis, Leptospirosis,* Canine Distemper, and endocrine disorders like diabetes are recommended.

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STATEMENT ON COMPETING INTEREST

The authors have no competing interest to declare.

AUTHOR'S CONTRIBUTION

Author MMM contributed to the development of the methodology, provided resources, performed the experiment, collected data, applied statistical techniques to analyze the data, wrote the original draft, and performed review and editing of the manuscript. Authors JAA and AMGAP conceptualized the study, designed the methodology, supervised the research activity, reviewed and edited the manuscript.

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