Quantitative intestinal ultrasonography for dogs’ parvoviral enteritis

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Abstract. The article is devoted to the quantitative characteristics of the small intestine wall layers echoicity of dogs with parvoviral enteritis, their ratio and comparison of the results with the index of healthy animals intestinal echoicity. The object of the study was 53 heterogeneous dogs of both sexes with confirmed polymerase chain reaction diagnosed with parvoviral enteritis at the age from 6 weeks to 7 months. The studies were carried out at the Pirogov Veterinary Center of Stavropol and SD&TVC at FSBEI HE "Stavropol State Agrarian University" on the SIUI Apogee 1100 scanner (Shantou Institute of Ultrasonic Instruments Co., Ltd., Guangdong, China) according to the generally accepted method using a multi-frequency linear sensor with a frequency of 7-12 MHz in B-mode. It was found that each layer of the jejunal wall (mucous, submucosal and muscular) of examined dogs was homoechoic and the intestine echoicity index of sick dogs increased for the mucous layer - 2.54 times, submucosal - 1.1, and muscle - in 1.3 times compared with healthy animals’ index. Intestinal echoicity index determination provides objective information about changes in the main ultrasound parameter during ultrasound of alimentary tract structures.

1. Introduction
In 1967 parvovirus was first identified as a cause of gastrointestinal and respiratory dogs’ disease and was subsequently named CPV-1. In 1978 outbreaks of an unknown contagious intestinal disease were reported the causative agent of which was isolated as a new species of the parvovirus family later named CPV-2 [1].

Currently, the most common manifestation of this disease is acute enteritis with nonspecific clinical signs at the initial stage such as anorexia, depression, stupor and fever. Later, more specific clinical signs develop: vomiting and small bowel diarrhea which can range from mucous to hemorrhagic. Due to the large loss of fluid and protein through the alimentary tract, dehydration and hypovolemic shock rapidly develop. Recorded abdominal pain is a feature of parvoviral enteritis and can be caused by acute inflammation of the stomach and intestines or less commonly intestinal obstruction. Damage to the intestinal tract increases the risk of bacterial translocation with subsequent coliform septicemia. This can lead to the development of a systemic inflammatory response which can progress to infectious toxic shock and ultimately lead to death [2].

According to Castro T X et al (2013) naturally infected with parvovirus dogs’ puppies had the main laboratory findings like leukopenia, lymphopenia, thrombocytopenia, hypoglycemia and hypoproteinemia [3], and Schoeman J P et al (2013) described additional laboratory diagnostic biomarkers for CPV enteritis: hypercoagulability, hypercortisolema, hypocrytrullinemia,
hypothyroxinemia, hypoalbuminemia, increased levels of C-reactive protein and tumor necrosis factor, and hypocholesterolemia [2]. Recently, cardiac biomarkers such as cardiac troponins and natriuretic peptides have been used as diagnostic and prognostic biomarkers of dogs with parvoviral enteritis [4].

Acute CPV-2 enteritis can occur among dogs of any breed, age or sex but puppies from 6 weeks to 6 months are more susceptible [2]. The CPV-2 virus spreads rapidly among dogs through the fecal-oral route (direct or nasal contact with vomit or feces from sick animals (indirect transmission). Severe viremia occurs 1-5 days after infection and clinical signs appear after 3-7 days of the incubation period [5] Parvovirus infects the embryonic epithelium of the intestinal crypts causing destruction of epithelium and villi. Normal cell turnover is damaged (usually 1-3 days in the small intestine) resulting in the appearance of shortened and atrophic villi. As a result of villous atrophy the small intestine loses its absorption capacity [2].

In the nosological structure of canine diseases in southern Russia diseases of the alimentary tract in dogs’ general pathology amounted to 29.8%. The share of parvoviral enteritis was 21% of alimentary tract pathology [6].

Standen N et al (2010) characterized alimentary tract structures of 40 puppies aged 6 to 24 weeks suffering from parvoviral enteritis on ultrasound examination. Sonographic findings included fluid-filled stomach, small and large intestine, generalized atony or weak peristaltic contractions, significant thinning and irregularity of the duodenum and jejunum mucosal surface. In addition, the authors found diffuse hypechoic spots in the duodenum and jejunum mucous layer, small intestinal corrugations were seen in the duodenum and jejunum. In addition, the authors note that sonographic changes severity is positively correlated with the clinical severity of patients [7].

Malancus R N et al (2017) performed ultrasound and endoscopic examinations among 133 dogs with gastrointestinal disorders to assess any significant correlations between ultrasound and endoscopic findings. The results confirm a significant correlation between the presence of diarrhea and increased colon wall thickness with P <0.5 and a relationship between increased colon wall thickness and loss of lamination at this level with P <0.5 [8]. Gaschen, L et al (2008) indicates that mucosal echoicity may be a better parameter for detecting inflammatory bowel disease than dogs’ bowel wall thickness with chronic diarrhea [9].

The lack of information in the available literature on the quantitative characteristics of dogs’ intestinal layers echoicity and their ratio in parvoviral enteritis served as the basis for these studies. The aim of the study was to provide a quantitative characterization of small intestine wall layers echoicity of dogs with parvoviral enteritis, their ratio and compare the results with healthy animals’ intestines index echoicity.

2. Materials and methods

The object of the study was heterosexual and heterogeneous dogs of both sexes with a confirmed diagnosis of parvoviral enteritis. The studies were carried out at the Pirogov Veterinary Center of Stavropol and SD and TVC at FSBEI HE "Stavropol State Agrarian University" in the period from September 2015 to October 2019. Totally 53 dogs were examined ranging in age from 6 weeks to 7 months. Ultrasound was performed on a SIUI Apogee 1100 scanner (Shantou Institute of Ultrasonic Instruments Co., Ltd., Guangdong, China) using a multi-frequency linear sensor from 7-12 MHz with the calibration setting of the ultrasound scanner on the scale of the instrument gain control (Gain) equal to 255 through the anterior and lateral abdominal wall with a series of transverse, longitudinal and oblique scanning sections with intestinal wall layers visualization. The echoicity of the intestinal wall was determined on archived digital ultrasound images of the intestine magnified threefold on a computer in a graphic editor Adobe Photoshop in black and white [10]. Two zones of each layer were assessed - the investigated and compared for which the investigated zone and the compared zone were outlined using the “lasso” tool, the histogram function was turned on while the numerical values of the parameters "mean value" and "deviation" were automatically displayed in the histogram window of the graphical editor, for the compared zone the "deviation error in the compared zone" was additionally determined for which this zone was divided into several sections, the deviation value in each section of this zone
was determined, the maximum deviation and minimum deviation in the compared zone or its sections were selected, then the deviation error was calculated in the compared zone according to the formula:

\[ E_{Dev2} = Dev_{max} - Dev_{min}, \]

where:
- \( E_{Dev2} \) – deviation error in the compared area;
- \( Dev_{max} \) – the maximum value of the deviation in the compared zone itself or its parts;
- \( Dev_{min} \) – the minimum value of the deviation in the compared zone itself or its parts.

Then the difference in deviations in the studied area and in the compared area was calculated using the formula:

\[ ΔDev = Dev_1 - Dev_2, \]

where:
- \( ΔDev \) – the difference in deviations in the investigated and compared areas;
- \( Dev_1 \) – deviations in the investigated area;
- \( Dev_2 \) – deviations in the compared area.

Then the deviation error in the compared zone was compared with the difference in deviations in the investigated and compared zone according to the formula:

\[ CEH = E_{Dev2} - ΔDev, \]

where:
- CEH – echo-homogeneity criterion of the investigated area;
- \( E_{Dev2} \) – deviation error in the compared area;
- \( ΔDev \) – the difference in deviations in the investigated and compared areas.

Provided that the CEH > 0, i.e. provided that the layers are homoechogenic, the coefficient or index of echoicity of the intestinal wall was calculated for which the fraction of echoicity of each layer of the intestinal wall was determined as the ratio of the obtained \( M_{brt} \) result for each individual layer of the jejunal wall (mucous, submucosal, muscular) as a percentage of the maximum reflection of the echo, expressed in the maximum value of the brightness of the pixels of an 8-bit image equal to 256 by the formula:

\[ PEL = (M_{brt} \times 100)/256, \]

where:
- \( PEL \) – separate layer echoicity proportion (mucous, submucous, muscle) in percent;
- \( M_{brt} \) – the average value of the brightness of the studied layer;
- 256 - the maximum value of the brightness of 8-bit image pixels: as a result, the index of echoicity of the jejunal wall IEsi was obtained:

\[ IEsi = PEL_m/PEL_s/m/PEL_mu, \]

where:
- \( IEsi \) - the index of small intestine wall echoicity;
- \( PEL_m \) - mucous layer echoicity proportion;
- \( PEL_{s/m} \) - submucosal layer echoicity proportion;
- \( PEL_{mu} \) - muscle layer echoicity proportion.

When conducting research and preparing an article for publication there was no conflict of personal, commercial, academic, intellectual and other interests.

To diagnose parvoviral enteritis intestinal epithelium scrapings were examined using a device for the quantitative detection of polymerase chain reaction (PCR) products in real time QuantiStudio 5, 96-well, 0.2 mL, Thermo Fisher Scientific, USA using a kit for detecting DNA viruses that cause parvoviral enteritis and panleukopenia “Fractal Bio” LLC, St. Petersburg, Russia.
Numerical data were processed using one-way analysis of variance and Student’s test for multiple comparisons, the dependence was revealed in the course of correlation analysis by calculating the linear Pearson coefficient in the Primer of Biostatistics 4.03 software for Windows on an IBM PC-compatible computer.

3. Research results
A retrospective analysis of small intestine wall echoicity was carried out on ultrasonographic images obtained from 53 dogs with a laboratory-confirmed diagnosis of parvoviral enteritis by staging a polymerase chain reaction (PCR) with the detection of viral DNA in scrapings of the intestinal epithelium. The small intestine ultrasound picture was characterized by weakening or absence of peristaltic contractions of the intestine, expansion of the intestinal cavity, the presence in it of a moderate amount of anechoic fluid content, thickening of the echographic layer corresponding to the outer part of the intestinal cavern and the border of the cavern with the mucosa and a decrease in the thickness of the mucous layer of the small intestine (figure 1).

![Figure 1](image1.png)

**Figure 1.** Left side: a fragment of healthy dog’s small intestine, longitudinal scan; right side: sonogram of longitudinal and transverse scans of the small intestine loops of an 18-week-old mongrel dog with parvoviral enteritis.

Each layer of the small intestine wall (mucous, submucous and muscular) in the examined dogs was homoechoic. The absolute values of small intestine wall layers echoicity in the form of the average brightness of the pixels of the layers are presented in the table 1. The "average" or Mbrt indicator is a weighted average level of brightness of image pixels which is automatically calculated by the computer by multiplying each brightness level by the number of pixels of this level and then dividing by the total number of brightness levels. This indicator depends on the settings of the scanner therefore it is important to obtain this information when calibrating the ultrasound scanner on the scale of the instrument gain (Gain) equal to 255 (the maximum possible value that allows you to obtain an image with a constant brightness value).

Characterizing the echoicity of each layer of dogs’ small intestine with parvoviral enteritis as a percentage of the maximum echo signal reflected in the maximum brightness of 8-bit image pixels an equal to 256, we obtained the following average results 39.37% (mucous), 61.57% (submucous), 45.56% (muscle) (figure 2).
Table 1. Absolute values of dogs’ small intestine wall echoicity with parvoviral enteritis (n = 53).

| Small intestine layers | Mucous | Submucous | Muscular |
|------------------------|--------|-----------|----------|
| Echocicity             |        |           |          |
| Average value of layers’ pixel brightness (m<sub>brt</sub>) | 100.78±10.24<sup>a</sup>/<sup>b</sup> | 12.03±1.39 | 157.61±14.21<sup>a</sup> | 116.63±12.40 | 13.93±2.99 |
| Scattering level of pixel brightness values | 12.03±1.39 | 157.61±14.21<sup>a</sup> | 18.47±4.55 | 116.63±12.40 | 13.93±2.99 |

Note: <sup>a</sup> - p≤0.01 comparing with muscular layer; <sup>b</sup> - p≤0.01 comparing with submucous layer.

Figure 2. Echocicity of the mucous, submucosal and muscle layers of the small intestine wall as a percentage of the maximum possible value of the reflected echo signal of dogs with parvoviral enteritis (n = 53) and healthy dogs (n = 86) (* cited from [11]).

Thus, the echocicity index of the small intestine wall of dogs with parvoviral enteritis averaged 39.37/61.57/45.56 (mucous, submucosal and muscle layers, respectively).

4. Discussion
As you know, the basic principle of ultrasound is to compare the ultrasound image of one tissue (area) under study with the image of another, i.e. characteristic of a particular body structure echocicity. In the main scanning mode - B-mode (from the English "Bright" - "brightness"), the ultrasound image is evaluated on the gray scale formed by the scanner based on the interaction of ultrasound with the tissues of the body. In this case, objects with different levels of brightness are visualized on the monitor. Since brightness is a subjective attribute of object properties perception, the analysis of changes detected during ultrasound examination based only on visualization can have different interpretations. A standard 8-bit image contains 256 different levels of brightness, the tool for its analysis is the brightness histogram which is a graph of the brightness gradient from zero (absolutely dim, black) to 255 (absolutely bright, white) and the number of image pixels that have the appropriate brightness. When analyzing the echocicity of dogs’ small intestine wall layers we obtained a quantitative indicator M<sub>brt</sub>, i.e.
the weighted average level of brightness of image pixels and based on these values the share of echoicity
of each layer was determined in the form of the ratio of $M_{brt}$ in percent to the maximum reflection of the
echo signal expressed in the maximum brightness of 8-bit image pixels equal to 256. The resulting
echoicity index of the dogs’ intestinal wall with parvoviral enteritis was compared with index previously
obtained in a study of healthy dogs [11], averaging 15.45 / 55.82 / 34.94 (mucous, submucous and
muscle layers, respectively). The echoicity of all layers of small intestine wall is higher in the group of
animals diagnosed with parvoviral enteritis. Thus, the echoicity of the mucous layer is 2.54 times higher,
the submucosa 1.1 times, and the muscular layer 1.3 times.

5. Conclusions
The intestinal echoicity index based on the ratio of the echoicity fractions of each layer from the
maximum brightness value of 8-bit image pixels, changes upwards in case of parvoviral enteritis of
dogs. The greatest increase in echoicity is observed in the mucous layer. Determination of the intestinal
echoicity index provides objective information about changes in the main ultrasound parameter in
pathological processes and conditions of the intestine and can be used for differential diagnostics during
ultrasound examination in veterinary gastroenterology.

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