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Abdominal ultrasonographic assessment in naturally acquired canine monocytic ehrlichiosis

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Abstract
Canine monocytic ehrlichiosis is an important rickettsial tick borne potentially fatal infectious disease of canids. Disease manifests in different phases with variety of clinical signs affecting various organs of body. The study was undertaken to assess ultrasonographic changes in the important abdominal organs like liver, gallbladder, spleen and kidneys following natural canine monocytic ehrlichiosis infection. Abdominal ultrasonography revealed hypoechogenicity of liver, spleen and kidney, gall bladder distension, hepatomegaly, splenomegaly and ascites.

Keywords: Dogs, *Ehrlichia canis*, ultrasonography, liver, spleen, kidney

1. Introduction
Canine monocytic ehrlichiosis (CME) is a tick borne disease caused by obligate intracellular gram negative bacterium, *Ehrlichia canis*. It is a multisystemic disease with multiple clinical manifestations (Harrus and Waner, 2011) [1]. Multiorgan dysfunction (involving the liver and spleen) is common in clinical cases of CME (Ganguly and Mukhopadhayay, 2008) [2]. Ultrasonography is a valuable imaging modality for evaluating abnormalities such as splenomegaly and alterations in the echogenicity and echotexture of parenchyma (Couto, 1992) [3]. Thus, ultrasound could be used as a valuable complementary tool in the diagnosis of CME, particularly during the subclinical period. Apart from other diagnostic procedures, ultrasonographic alterations of the liver and spleen in tick-borne intracellular haemoparasitic diseases can be utilized as a further diagnostic aid (Sarma *et al.*, 2014) [4]. Thus, the present investigation was carried out to assess ultrasonographic changes in the important abdominal organs like liver, gallbladder, spleen and kidneys in dogs naturally affected with CME.

2. Materials and Methods
The present investigation was carried out in the 34 dogs diagnosed positive for CME by nested PCR presented to Veterinary Clinical Complex (VCC), West Bengal University of Animal & Fishery Sciences (WBUAFS), Belgachia, Kolkata. In this study, the Mindray diagnostic ultrasound system (Model: DC-3Vet), Shenzhen Mindray Bio-Medical Electronics CO. LTD., was used for per-cutaneous scanning of Liver, Spleen and Kidney. The images were acquired with 5-7.5 MHz 2D curvilinear transducer.

2.1 Site preparation
For ultrasonographic examination, the dogs were restrained in lateral recumbency (without using any anaesthesia or sedatives) to scan the desired organs. The entire abdominal area was shaved, and coupling gel was applied over the transducer as well as the area of interest before the sonogram to ensure an intimate contact between the scan head and the body surface. The dogs were left undisturbed throughout the ultrasonography procedure in order to ensure optimal results.

2.2 Ultrasonography procedure
For hepatic ultrasonography the transducer was placed on the midline at the xiphoid cartilage, and a longitudinal image was obtained aligning the transducer plane parallel to the long axis of the animal and tilting the transducer in a cranial direction. Angling the transducer plane to the right or left allows lateral imaging.

Turning the transducer 90° permits a transverse imaging. The entire liver was examined by angling the transducer steeply from a craniodorsal direction to cranioventral direction. The gall bladder and the porta hepatis are useful landmarks for ultrasonography of the liver.

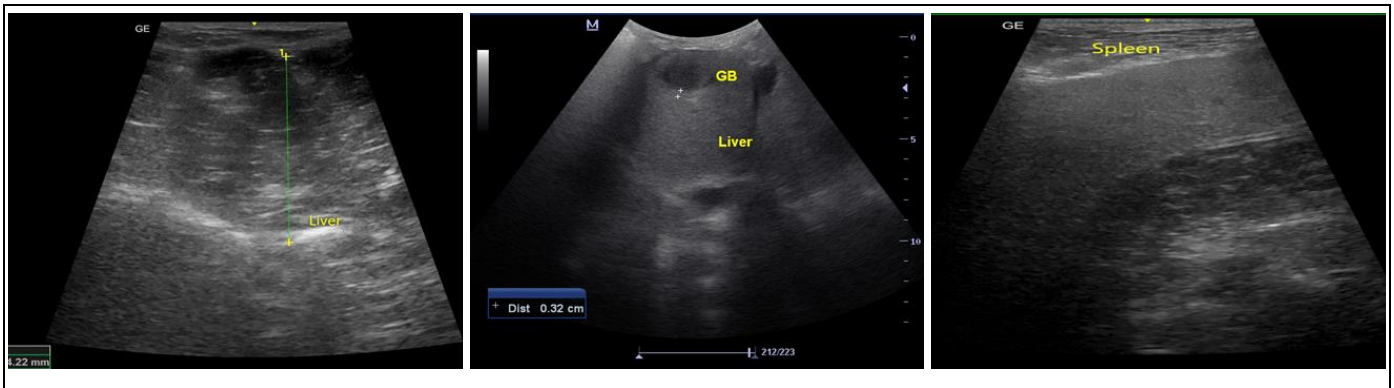
The size of gall bladder on ultrasonography was found variable depending on whether or not animal has recently eaten. For right transverse oblique screen of gall bladder, the transducer was placed on the right side approximately 6-8 cm cranial to xiphoid and 4-5 cm dorsal to the sternum. The transducer was angled towards midline between costal cartilages. For left transverse oblique scan of the gall bladder, the transducer was placed on right 10^{th}-11^{th} inter costal space, 5-10 cm ventral to the spine. Sonograms were analyzed for liver size, shape, contour and its internal architecture, as well as changes in echogenicity and intensity (anechoic/ normoechoic/hypoechoic/hyperechoic), gall bladder (for size, shape, wall and its contents) and the presence or absence of free peritoneal fluid.

The dogs were either placed in dorsal or right lateral recumbency for the examination of the spleen. The spleen was scanned from a mid-line sagittal position and the transducer was angled to the left side of abdomen. The spleen lies just caudal to the liver and lateral to the stomach. The location of the spleen varies depending on the gastric contents. The head of the spleen is located under the border of the rib cage, while the body and tail of the spleen extend along the left abdominal wall or across the ventral abdomen. The spleen lies ventral or lateral to the left kidney. When it is enlarged it may cross the ventral midline and extends to the bladder region. On ultrasonography normal spleen has a dense, homogeneous, granular, speckled echotexture and is more echogenic than the liver and kidney. Spleen was examined for echotexture (homogeneous or heterogeneous), echogenicity (hypo, hyperechoic, or mixed), size (increased, reduced, or normal), and borders (rounded or sharp).

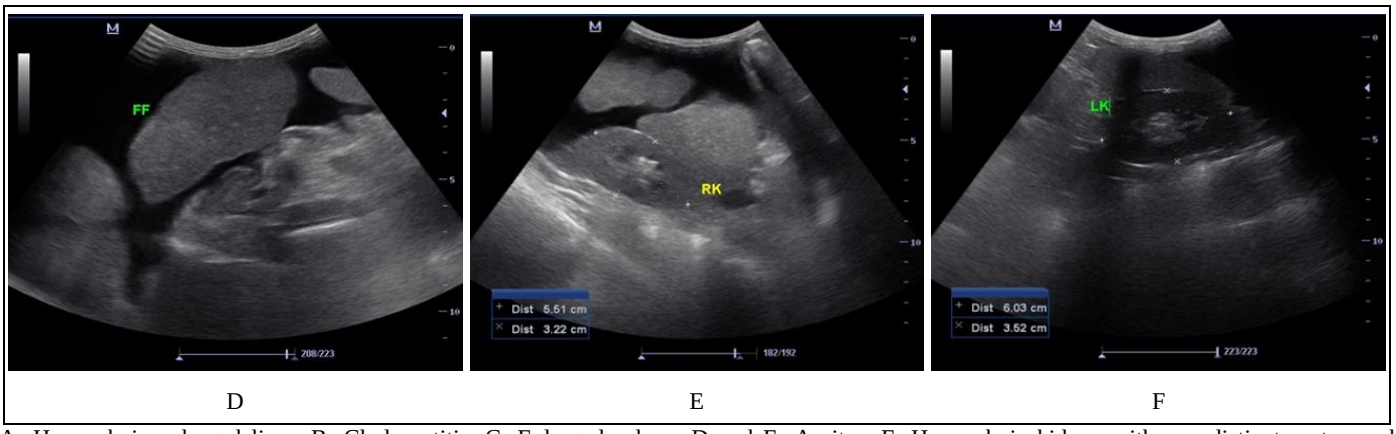
For visualization of the kidneys, the dogs were placed in lateral recumbency (left lateral recumbency for the right kidney and right lateral recumbency for the left kidney). In dogs, the left kidney can be evaluated through the body of spleen, which provides a good acoustic window. Longitudinal and transverse scans of the whole kidney should be conducted. The renal cortex and medulla, as well as the region of the renal pelvis and renal vessels, may be seen in the transverse plane. The right kidney is more cranial and dorsal and usually in contact or close proximity with the hepatic parenchyma at the level of caudate lobe. Right-lateral intercostal approaches (between the 11^{th} and 12^{th} intercostal gaps) are frequently required for complete visualization of the right kidney. Lateral recumbency helps to avoid bowel gas interference while examining the kidneys. The size, shape, contour, echogenicity, and distinction between the cortex and medulla of the kidneys were all assessed.

3. Results and Discussion

Hepatic ultrasonography findings revealed hypoechogenicity of liver, inflammatory changes and hepatomegaly. Out of 34 CME-affected dogs, hypoechogenicity of liver was recorded in 15 (44.11%) cases while hepatomegaly was observed in 10 (29.41%) cases. Gall bladder distension was recorded in 2 (5.88%) cases with presence of sludge/clear bile. Ascites was observed in 3 (8.82%) cases. Splenic ultrasonography findings revealed hypo echogenicity, splenomegaly along with inflammatory changes. Out of 34 CME-affected dogs, hypo echogenicity of spleen was recorded in 11 (32.35%) cases while splenomegaly was observed in 7 (20.58%) cases. Ultrasonography findings of kidney revealed normal physiological size of kidney, hypo echogenicity, as well as inflammatory changes. Out of 34 CME-affected dogs, hypo echogenicity of kidney was observed in 5 (14.70%) cases, while poorly visualized renal architecture with non-distinct cortex and medulla was seen in 2 (5.88%) cases. The ultrasonography findings of present investigation are in harmony with the previous authors (Singh, 2020; Rao *et al.*, 2020; Sarma *et al.*, 2014; Behera *et al.*, 2012; Kumar, 2004) [5-8]. Sakuma *et al.* (1987) [17] also reported hypoechogenicity of liver along with gall bladder distension in mixed parasitic infections. Additionally Angkanaporn *et al.* (2022) [9] reported hyperechogenicity of the liver while kidney showed a hyperechoic echotexture compared with the spleen. In case of tick-borne intracellular diseases, hepatomegaly may be attributed to passive congestion, reticuloendothelial hyperplasia, or infiltrative diseases mediated through cytokines (Meyer and Twedt, 2000) [10]. Splenomegaly results from reactive lymphoid hyperplasia and extramedullary hematopoiesis (Egenvall *et al.*, 2000) [11] and occur in approximately 20% to 25% of cases (Saito and Walker, 2016) [12]. In previous studies spleen has been shown to be a major reservoir of *E. canis*, probably owing to the abundance of hosting macrophages in this organ. It is also the last organ to shelter the pathogen before it is eliminated (Harrus *et al.*, 2004; Harrus *et al.*, 1998) [13, 14]. The USG change in gall bladder included distention with presence of sludge/clear bile which might be attributable to anorexia (Kumar, 2004) [8]. Renal insufficiency as a consequence of CME has been linked to immune-complex-mediated, resulting in glomerulonephritis and proteinuria (Harrus *et al.*, 2002) [15]. The loss of architectural details is an important feature of chronic renal disease and arises due to a gradual loss of nephrons as time progresses (Felkai *et al.*, 1992) [16].



A B C



A: Hypoechoic enlarged liver, B: Cholecystitis, C: Enlarged spleen, D and E: Ascites, F: Hypoechoic kidney with non-distinct cortex and medulla

Fig 1: Ultrasonographic changes in CME

4. Conclusion

The overall impression of the present investigation was suggestive of inflammatory changes in the liver, spleen and kidney. Thus, along with other laboratory tests, ultrasonography serves as a valuable non-invasive diagnostic imaging technique for determining clinical severity, organ involvement, and monitoring therapy in CME cases.

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