

## Ultrasonographic examination of the spleen in newborn calves

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### Abstract

The ultrasonographic appearance, location and size of the spleen in 40 healthy male and female Holstein calves are described. The intercostal spaces of the left thoracic wall were scanned with 5.0 MHz linear transducer in lateral recumbent calves. The appearance of the splenic parenchyma, the position of the ultrasonographically visible dorsal and ventral margins of the spleen. The distance, thickness of the spleen and the diameter of the splenic vessel were determined. The spleen could be visualised in at least one examined position and it always lies between the abomasum and abdominal wall. The spleen bordered the lung dorsally and was located adjacent to the left abdominal wall in the last intercostal space. The spleen had an echogenic capsule and its parenchyma showed a homogenous fine echotexture/echo pattern throughout the whole visible parts of the spleen. The splenic vessel was seen within the parenchyma. The visible dorsal margin of the spleen ran from cranioventral to caudodorsal. The distance from the dorsal margin of the spleen to the midline of the back was largest in the 7th intercostals space ( $24.37 \pm 1.69$  cm) and smallest in the 12th intercostal space was  $6.80 \pm 0.68$  cm. The size of the spleen was smallest in the 8th intercostalspace ( $1.97 \pm 0.99$  cm) and greatest in the 11th intercostalspace ( $4.13 \pm 1.14$  cm). The thickness of the spleen ranged from  $11.32 \pm 2.60$  in 7th to  $9.51 \pm 4.16$  mm in the 12th intercostal space.

**Keywords:** Calve; spleen; ultrasonography; normal findings

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### Introduction

Ultrasonography is used routinely to evaluate spleen in cattle, horse and goat, however, there is no report in calves. There is no-data about location and topography of spleen in neonatal calves. In mature cattle, the flat oblong spleen is situated over the craniodorsal part of the rumen, against the left half of the diaphragm, and is attached to both organs. Its upper end lies under the dorsal ends of the last few ribs, and its axis extends ventrally, with a slight cranial inclination, across the line of the ribs to end in the region of the seventh costochondral joint (Dyce et al.,

2010). The hilus of the spleen is confined to the dorsocranial angle of the medial side and contain splenic artery and vein (Dyce et al., 2010). The spleen is a storage area for red blood cells and filters blood through a sinusoidal system (Rowen et al., 2009). It has additional functions in lymphopoiesis, antibody production, hemopoiesis under conditions of increased demand for blood cells by colonization of sinusoids with pluripotential stem cells, phagocytosis and opsonization of pathogen, participation in the recycling and metabolism of iron. A variety of changes occurs in the spleen response to systemic states (Douglas et al., 2010). Systemic inflammations cause a regular and

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fairly predictable pattern of response in the spleen. In septicemia and following injection of endotoxin or gram-negative bacterins, there is rapid accumulation of neutrophils in the splenic marginal zone and surrounding area of sinus (Jubb et al., 2007). Splenomegaly with complete destruction of splenic function is virtually symptomless, especially if the involvement occurs gradually, and in most cases clinical signs are restricted to those caused by involvement of other organs. Rupture of a grossly enlarged spleen may cause sudden death due to internal haemorrhage. This is sometimes the cause of death in bovine viral leukosis or equine amyloidosis. Moderate degrees of splenomegaly occurs in many infectious diseases, especially salmonellosis, anthrax, babesiosis, neoplasms of the spleen in lymphosarcoma, splenic abscess, perforation by a foreign body in the reticulum, equine infectious anemia and diplococcus septicemias in calves, and in some noninfectious diseases such as ascoptertoxicity in sheep (Radostits et al., 2007). There were no published data, characteristic sings of splenic disease and specific methods for examination of this organ in calves. It cannot be palpated externally and there is no specific laboratory test for determining splenic disease. The goal of this study was to describe the ultrasonographic appearance, size and location of the spleen and associated blood vessel in 40 healthy calves.

## Materials and Methods

Forty clinically healthy Holstein dairy calves (20 male & 20 female) below one week old and weighed  $40 \pm 5$  kg were examined. All of the calves were born with eutocia and fed 10 to 12% of body weight colostrum 4 hours postpartum. Each calf was placed in individual pen with straw bed. For confirmation of healthy status vital signs (body temperature, heart rate, and respiratory rate), complete blood cell count, PCV and total protein, hydration status, suckling reflex and thorough clinical examination were done.

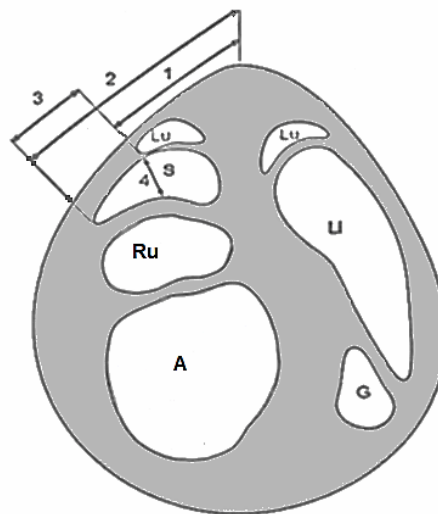
### Ultrasonographic examination of the spleen

A real time ultrasound machine (ECM AL, Agrosan, France) with a 5.0 MHz linear transducer was used to examine the spleen of non-sedated calves, restrained physically in lateral recumbency (Fig. 1). To calculate the measurement of the spleen in each intercostal space, we followed the method described for cattle (Sicher, 1995). After clinical examination, left thoracic wall was clipped from the caudal border of the shoulder to just caudal to the last rib and from dorsal midline to the linea alba. Each intercostal space and the region caudal to the last rib were examined from dorsal to ventral with the transducer held parallel to the ribs. First the appearance of the splenic capsule and

parenchyma were assessed subjectively. The size and location of the spleen were then calculated using the values determined for the position of the dorsal and ventral margins of the spleen. Figure 2 shows how the measurements were made in each intercostal space. They included determination of the dorsal visible and ventral margins, size and maximal visible thickness of the spleen. Measurements were made at maximum inspiration. The positions of the dorsal visible and ventral margins of the spleen were determined relative to the midline of the back. The size of the spleen was calculated by



**Fig.1: Physical restraint of a calf for ultrasonographic examination.**



**Fig. 2: Schematic representation of determination of the position and size of the spleen in calves on a cross section in the 11th intercostal space. Lu Lung, Li Liver, G Gallbladder, S Spleen, Ru Rumen, A Abomasum, U Uterus, 1 Distance between dorsal midline and dorsal margin of the spleen, 2 Distance between dorsal midline and ventral margin of spleen, 3 Size of spleen, 4 Thickness of spleen.**

subtracting the value for the dorsal visible margin of the spleen from that for the ventral margin. The diameter of blood vessel in the spleen was measured electronically using the two calipers.

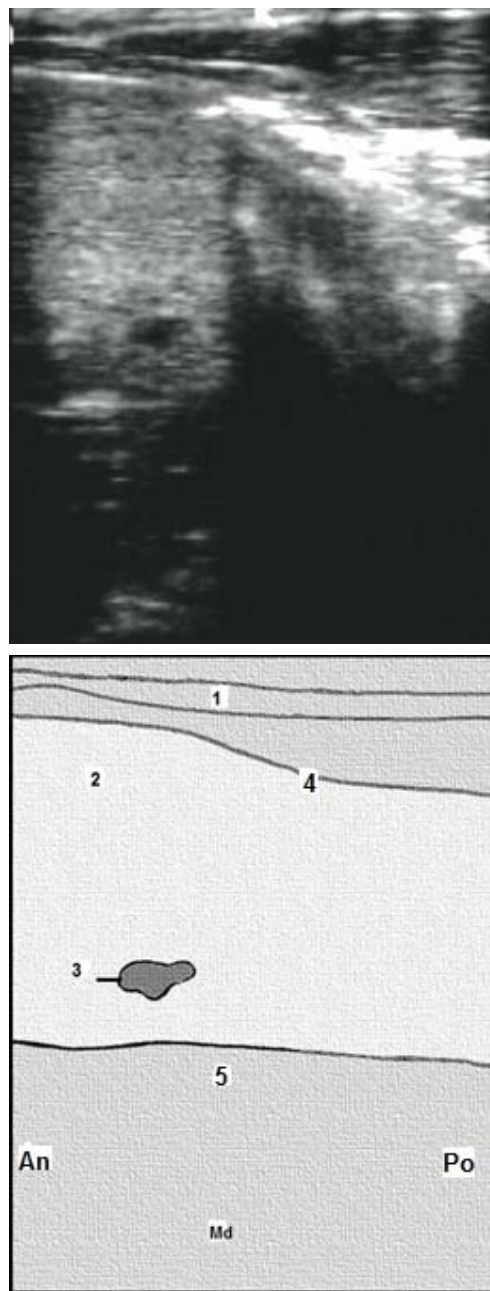
## Results

The spleen was seen in the 7th, 8th, 9th, 10th, 11th, and 12th intercostal spaces in 72.5, 95, 97.5, 100, 100 and 100% calves, respectively (Table 1). Mostly it was located between the abomasum and left abdominal wall in all calves. Dorsally, the parietal surface of the spleen was in contact with the lung and the most dorsal part was partially covered, and in the last intercostal space, and lay against the left abdominal wall. There was movement of the spleen relative to the peritoneum in synchrony with respiration. The spleen had an echoic capsule and the parenchymal pattern consisted of numerous weak echoes distributed homogenously over the entire visible area of the spleen (Fig. 3). The vessel of the spleen appeared as anechoic circular to oval or elongated structures. They had a diameter of 0.60 to 0.92 cm and seen better in 11th intercostal space (Fig. 3). The distance between the dorsal margin of the spleen and the midline of the back was greatest in the 7th intercostals space ( $24.37 \pm 1.69$  cm) (Table 1). This distance decreased caudally and was only  $6.80 \pm 0.68$  cm in the last intercostal space. The ventral margin of the spleen had a similar course. It was greatest in the 7th intercostal space ( $26.53 \pm 1.68$  cm) and shortest in the region of the last intercostal space ( $9.52 \pm 1.01$  cm). The size of the spleen was smallest in the 8th intercostal space ( $1.97 \pm 0.99$  cm) and largest in the 11th intercostal space ( $4.13 \pm 1.14$  cm). The thickness of the spleen ranged from  $11.32 \pm 2.60$  mm in 7th to  $39.51 \pm 4.16$  mm in the 12th intercostal space. The data of females and males calves are shown in Table 2 and 3.

## Discussion

Ultrasonography is an ideal, non-invasive method to examine the bovine spleen (Braun, 2003). Braun et al. (2006) described the ultrasonographic appearance, location and size of the spleen in milking cows with standing position. In small ruminant, ultrasonographic appearance, location and size of the spleen in 30 healthy female Saanen goats with standing position have been reported (Braun et al., 2010). The spleen volumes in horses were estimated by Nolen-Walston et al. (2011). All of these studies were performed in adult animals in standing position. In this study, the spleen of neonate calves in lateral recumbent position was evaluated. Most important diseases in neonatal calves that may lead to changes in splenic size are septicemia and dehydration, and in majority of cases both of them lead to recumbency in animal, so in most cases of

septicemia or dehydration ultrasono-graphic examination of spleen in calves with standing position are impossible. Because topography of abdominal organs may change in different position, for measuring normal splenic index, it is better to examine calves in



**Fig. 3:** Ultrasonogram and schematic representation of the splenic parenchyma of a 6 day old Holstein dairy calve. A 5.0-MHz linear transducer was placed on the left thoracic wall in lateral recumbency position, parallel to the ribs in the 11th intercostal space. 1 – thoracic wall; 2 – spleen; 3 – spleen vessel; 4 – spleen capsule; 5 – abomasum, An – anterior; Po – posterior; Md – medial.

**Table 1: Results of ultrasonographic examination of the spleen in 40 Holstein dairy calves (Mean  $\pm$  SD)**

Variable	Location					
	12th intercostal space	11 <sup>th</sup> intercostal space	10th intercostal space	9th intercostal space	8 <sup>th</sup> intercostal space	7 <sup>th</sup> intercostal space
Number of calves	40	40	40	39	38	29
Dorsal margin of spleen (cm)	(6.80 $\pm$ 0.68) 5.50 - 8.0	(9.46 $\pm$ 1.21) 8.0 - 12.0	(13.65 $\pm$ 1.85) 11.0 - 17.0	(18.07 $\pm$ 1.97) 13.0 - 22.0	(21.60 $\pm$ 1.56) 19.0 - 24.0	(24.37 $\pm$ 1.69) 21.0 - 28.0
Ventral margin of spleen (cm)	(9.52 $\pm$ 1.01) 7.50 - 12.0	(13.61 $\pm$ 1.27) 11.0 - 16.0	(17 $\pm$ 1.73) 13.50 - 20.0	(19.96 $\pm$ 1.61) 17.0 - 24.0	(23.56 $\pm$ 1.59) 20.0 - 26.0	(26.53 $\pm$ 1.68) 23.0 - 30.0
Size of spleen (cm)	(2.71 $\pm$ 0.88) 1.0 - 4.0	(4.13 $\pm$ 1.14) 2.0 - 6.50	(3.37 $\pm$ 1.24) 2.0 - 7.0	(2.39 $\pm$ 1.03) 1.0 - 6.0	(1.97 $\pm$ 0.99) 1.0 - 4.0	(2.15 $\pm$ 0.74) 1.0 - 4.0
Thickness of spleen (mm)	(39.51 $\pm$ 4.16) 30.80 - 45.60	(39.2 $\pm$ 6.33) 29.20 - 48.20	(31.59 $\pm$ 5.24) 22.10 - 44.0	(24.42 $\pm$ 4.34) 15.50 - 32.0	(16.26 $\pm$ 4.40) 10.0 - 26.0	(11.32 $\pm$ 2.60) 6.40 - 15.40

Ranges are in brackets

**Table 2: Results of ultrasonographic examination of the spleen in 20 males Holstein dairy calves (Mean  $\pm$  SD)**

Variable (male)	Location					
	12th intercostal space	11 <sup>th</sup> intercostal space	10 <sup>th</sup> intercostal space	9th intercostal space	8th intercostal space	7 <sup>th</sup> intercostal space
Number of calves	20	20	20	19	18	12
Dorsal margin of spleen (cm)	(6.72 $\pm$ 0.71) 5.0 - 8.0	(9.45 $\pm$ 1.22) 8.0 - 12.0	(14.30 $\pm$ 1.97) 11.0 - 17.0	(18.57 $\pm$ 1.82) 15.0 - 22.0	(21.72 $\pm$ 1.55) 19.0 - 25.0	(24.83 $\pm$ 1.24) 22.0 - 26.0
Ventral margin of spleen (cm)	(9.22 $\pm$ 1.14) 7.0 - 11.0	(13.90 $\pm$ 0.95) 12.0 - 15.0	(17.17 $\pm$ 1.83) 13.50 - 20.0	(20.60 $\pm$ 1.72) 17.0 - 24.0	(23.58 $\pm$ 1.69) 21.0 - 26.0	(26.79 $\pm$ 1.68) 23.0 - 29.0
Size of spleen (cm)	(2.50 $\pm$ 0.77) 1.0 - 3.50	(4.42 $\pm$ 0.94) 3.0 - 6.0	(2.92 $\pm$ 0.82) 2.0 - 5.0	(2.02 $\pm$ 0.42) 1.50 - 3.50	(1.88 $\pm$ 0.91) 1.0 - 4.0	(2.04 $\pm$ 0.87) 1.0 - 3.50
Thickness of spleen (mm)	(40.58 $\pm$ 3.49) 33.60 - 46.0	(38.80 $\pm$ 6.19) 29.50 - 46.40	(31.92 $\pm$ 6.02) 22.10 - 44.0	(22.84 $\pm$ 4.53) 14.0 - 30.0	(14.97 $\pm$ 3.53) 10.0 - 21.90	(10.97 $\pm$ 2.82) 6.40 - 15.40

Ranges are in brackets

**Table 3: Results of ultrasonographic examination of the spleen in 20 females holstein dairy calves (Mean  $\pm$  SD)**

Variable (female)	Location					
	12th intercostal space	11 <sup>th</sup> intercostal space	10th intercostal space	9th intercostal space	8th intercostal space	7th intercostal space
Number of calves	20	20	20	20	20	17
Dorsal margin of spleen (cm)	(6.87 $\pm$ 0.68) 5.0 - 8.0	(9.47 $\pm$ 1.22) 8.0 - 12.0	(13.0 $\pm$ 1.51) 11.0 - 16.0	(17.60 $\pm$ 2.04) 13.0 - 20.0	(21.50 $\pm$ 1.60) 19.0 - 24.0	(24.05 $\pm$ 1.92) 21.0 - 28.0
Ventral margin of spleen (cm)	(9.85 $\pm$ 0.81) 9.0 - 11.0	(13.32 $\pm$ 1.50) 11.0 - 16.0	(16.82 $\pm$ 1.67) 14.0 - 20.0	(20.35 $\pm$ 1.60) 17.0 - 23.0	(23.55 $\pm$ 1.54) 20.0 - 26.0	(26.35 $\pm$ 1.71) 24.0 - 30.0
Size of spleen (cm)	(2.97 $\pm$ 0.93) 1.0 - 5.0	(3.85 $\pm$ 1.27) 2.0 - 6.50	(3.82 $\pm$ 1.42) 2.0 - 7.0	(2.75 $\pm$ 1.31) 1.0 - 6.0	(2.05 $\pm$ 1.08) 1.0 - 4.0	(2.23 $\pm$ 0.73) 1.0 - 4.0
Thickness of spleen (mm)	(38.46 $\pm$ 4.68) 30.80 - 45.60	(39.64 $\pm$ 6.59) 29.20 - 48.20	(31.25 $\pm$ 4.47) 21.90 - 41.30	(25.92 $\pm$ 3.65) 17.20 - 32.0	(17.43 $\pm$ 4.85) 10.60 - 26.0	(11.57 $\pm$ 2.48) 7.0 - 16.0

lateral recumbency. Spleen is the organ responsible for lymphopoiesis, antibody production and red blood cells storage (Douglas et al., 2010). In anemia, dehydration and infectious disease the splenic size could change (Radostits et al., 2007; Nolen-Walston et al., 2011). The spleen cannot be palpated externally. Ultrasonography is an ideal, useful and non-invasive method for examining the bovine spleen (Braun and Sicher, 2006). All the compartments of the ruminant stomach develop from a primordium and relative sizes of the four compartments change with age (Sisson and Grossman, 1975). Thus topography of abdominal organs adjacent

to spleen (rumen, abomasum, intestine) is different in calves compare to cows, so ultrasonographic reference of spleen in cow is useless for neonatal calves. To compare pathologic conditions with normal situations, it is essential to have normal ranges of ultrasonographic indexes of spleen. This study was conducted to standardize ultrasonography indexes of spleen in calves. Ultrasonographic examination of calf's spleen was straightforward. spleen was located adjacent to the costal part of the left abdominal wall, it could be clearly visualized in all the calves from the 7th intercostal space to the 12th intercostal space and was not seen in

region caudal to the last rib. The splenic parenchyma was best evaluated in the 10th to 12th intercostal spaces as the organ was seen in almost all calves in this area. The ultrasonographic appearance of the splenic parenchyma was similar to that reported for small animals (Radostits et al., 2007), horses (Reef, 1998) and cattle Braun and Sicher (2006) and Sicher (1995). It consisted of numerous small echoes homogeneously distributed over the entire area of the organ as for liver ultrasonography. However, the two organs can be easily differentiated because of their different anatomical locations. The splenic vasculature consists of the splenic artery and the splenic vein (Dyce et al., 2010), but we cannot differentiate them on routine splenic scans. The hilus of the spleen is not visible on ultrasonograms because it is superimposed by lung. Ultrasonography is a useful tool for the non-invasive examination of the spleen. Its appearance and size in healthy calves can serve as reference values for the diagnosis of pathological changes in the spleen. Splenic lesions diagnosed via ultrasonography in cattle have been associated with traumatic reticuloperitonitis (Braun et al., 1993; Braun et al., 1998; Braun, 2003). Although in dogs, tumours, haematoma, atrophy and hypertrophy of the spleen as well as splenic torsion have been diagnosed via ultrasonography (Dyce et al., 2010). Therefore possibility of ultrasonographic changes of spleen and other positions or methods in diseases of calves could be evaluated in future.

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