



ISSN (E): 2277- 7695

ISSN (P): 2349-8242

NAAS Rating: 5.03

TPI 2020; 9(5): 250-255

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www.thepharmajournal.com

Received: 14-03-2020

Accepted: 16-04-2020

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Cadaveric, organ bath and real time ultrasonographic studies on spleen in calves

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Abstract

Three phase study *i.e.*, cadaveric, *in vitro* organ bath and real time ultrasonography was conducted on neonatal (G1), pre-ruminant (G2) and ruminant calves (G3) to develop a baseline data for normal calf spleen. Cadaveric study showed spleen was placed obliquely on left side of abdomen. In G1 animals caudodorsal base was distal to T₁₁ to T₁₃ vertebrae spreading medially to these vertebrae across 11th to 12th intercostal spaces (ICS). Spleen was 8-9 cm in length from base to apex and its width at broadest part was 4.5- 5 cm. visceral surface of spleen in G3 animals was associated more with developing rumen than abomasum. Splenic parenchyma was isoechoic granular devoid of vasculature during organ bath studies. During real time ultrasonographic studies acoustic window for Spleen was seen from 6th to 11th intercostal spaces (ICS) of left abdomen in the animals of G1, 8th to 12th ICS in G2 and G3 animals with dorsal and ventral margins following almost the same pattern of G1 animals. Splenic parenchyma was uniformly echogenic in nature. Capsule appeared as a fine hyper echoic line. In the animals of G3, spleen extended ventrally far enough to contact the liver. Spleen was of the largest size at 6th, 7th and 8th ICS in the animals of G1, G2 and G3 respectively. Thickness of the spleen increased with increase in age of calves from G1 to G2. A fair resemblance of cadaveric, organ bath and RTU studies was recorded.

Keywords: Calf, organ bath study, real time ultrasonography, spleen, surgery

Introduction

Spleen is a lymphoid organ associated with the circulatory system It has important immunologic functions, besides being a storage area for red blood cells and carry destruction of worn-out erythrocytes. In ruminants, it adheres closely to the rumen. Spleen is a useful organ in growing animals, although not essential in the adults (Frandsen, *et al.*, 2009)^[9]. Calves, prominently neonatal ones can get acutely infected with a diverse species of bacteria especially Salmonella (Anderson *et al.*, 2001)^[1]. However older calves respond with chronic wasting, partial anorexia, debility fever, dullness, and scours that often contain increased mucus (Sojka and Field, 1970)^[15]. The spleen is not easily accessible for clinical examination by palpation or percussion, because of its topographic location under costal part of the abdominal wall. It can neither be examined by rectal palpation nor radio graphed, thus making the diagnosis of splenic conditions very difficult (Braun and Sicher, 2006)^[59]. This comprehensive study on cadaveric, organ bath and real time ultrasonographic view of spleen in healthy calves was conducted to develop a baseline data for determining its pathology in calves.

Materials and Methods

This comprehensive study of cadaveric, invitro organ bath and real-time ultrasonographic (RTU) was conducted on calves of different age groups, which were distributed in three groups designated as group 1(G1), group 2(G2) and group 3(G3) depending upon their age as follows.

Group	Category of the calf	Age	Number of animals	
			Cadaveric and In-vitro	RTU
G1	Neonatal calves	Birth to 1 month	02	06
G2	Pre-ruminant calves	From 1 month upto 3 months	02	06
G3	Ruminant calves	From 3 months upto 9 months	02	06

Cadaveric and *in vitro* study or water bath study was conducted in six (6) cadavers of different age group calves.

All the anatomical features of spleen like position and location were recorded and documented. Water bath ultrasonographic study of spleen was performed to record its echo texture. The morbid material collected from the six cadavers was put in a water bath at 37°C temperature and subjected to repeated ultrasonography by a 7.5 to 12 MHz linear transducer. Real time ultrasonography (RTU)/ *In vivo* study was carried on 18 healthy calves belonging to three

different age groups as depicted in the table.

A real time B-mode ultrasound machine Esoate My Lab 40 Vet (Plate 1A) fitted with a linear and sector transducer of 3.5-12 MHz frequency (Plate 1B) was used in both water bath and RTU studies. Copious amount of sonography transmission gel "Aquason 2000" (Plate 1C) was applied on the body surface before fixing of the transducer on live animals.

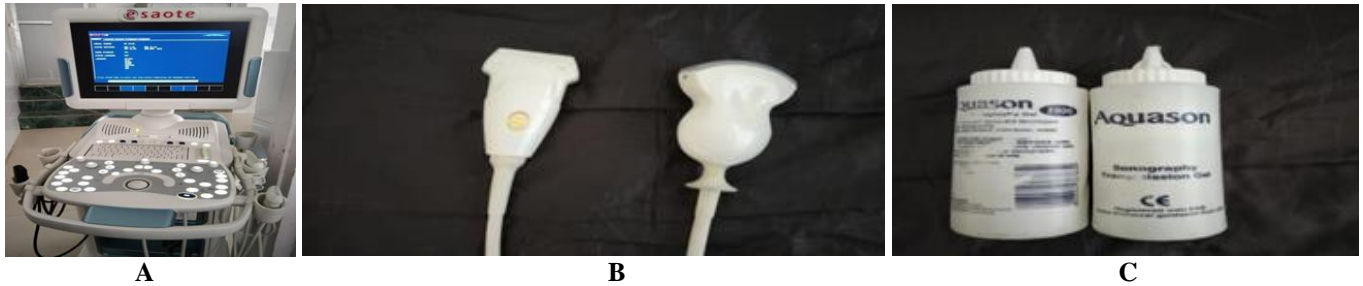


Plate 1: Ultrasound machine Esoate My Lab 40 Vet A, with curvilinear and linear transducers B, and coupling gel C

Calves were secured in standing position without any sedation. The abdominal area of each calf, from tuber-coxae to 5th intercostal space in horizontal plane and from dorsal

midline of vertebral column to linea alba on both sides in vertical plane were shaved and first cleaned with tap water followed by isopropyl alcohol (Plate 2). Calves in



Plate 2: Animal preparation A, and restrain during Real time ultrasonography B.

In RTU were examined with a 3.5 MHz curvilinear transducer followed by 10-12MHz linear transducer. The maximum depth of field for this transducer was 7-12 cm.

Sonographic parameters of calf spleen in RTU studied were: a) acoustic window, b) echogenicity patterns, c) location of the organs, and d) biometry of the organs. Transducer placement loci for identification of proper acoustic window to obtain the optimal images were recorded. Dimensions of spleen mean \pm SE were measured and recorded. The positions of the dorsal and ventral margins were measured in relation to the midline of the back to calculate their size. The effective length of spleen was calculated by measuring the distance between the transducer fixing sites depicting visible margins of the organs along the long axis of organs through intercostal spaces, and the transducer fixing sites where the margins of the organs became invisible. The data was statistically analyzed by SPSS software using "one way ANOVA" for comparing the means and calculating the significance of the data. The means were subjected to DMRT to compare groups.

Results

Cadaveric Study

In Group 1 (G1) animal's spleen was seen placed obliquely on left side of abdomen. Base of the spleen was located caudodorsally distal to T₁₁ to T₁₃ vertebrae spreading medially to these vertebrae across 11th to 12th intercostal spaces (ICS). The apex was cranioventrally located medial to distal third of 6th to 7th ICS (Plate 3). Spleen was 8-9 cm in length from base to apex. Its width at broadest part was 4.5- 5 cm. Parietal surface at apex was closer to diaphragm as compared to rest of the surface. Spleen lung interface at apex was seen ventral to T6 vertebra corresponding 5th ICS. Ventromedial aspect of spleen at its apex and part of body adjacent to it was parallel to cardia of abomasum. Dorsomedial part of spleen was lateral to reticulum and rumen. In Group 2 (G2) and Group 3 (G3) animals apex was placed from 6th to slightly behind 7th ICS. Visceral surface of spleen in G3 animals was associated more with developing rumen than abomasum. Average length of spleen in G2 & G3 animals was about 12 and 15.5 cm. while width at broadest part was 5 and 7 cm respectively.

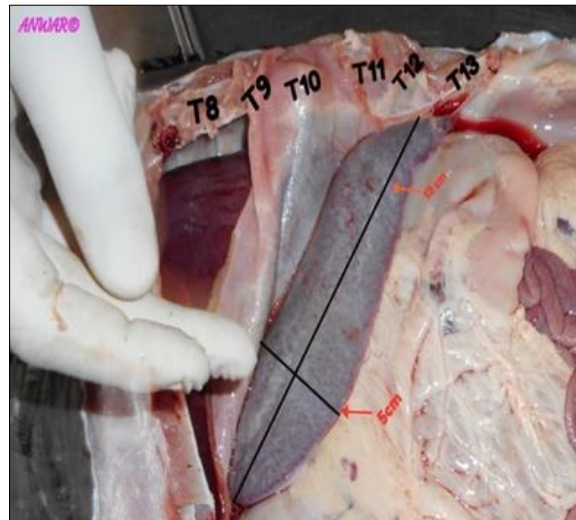


Plate 3: Cadaveric appearance of Spleen

In vitro organ bath study

12 MHz linear transducer used for scanning spleen both for its longitudinal and transverse views was found appropriate. The

splenic parenchyma showed isoechogenic granular pattern with an enveloping thick hyperechogenic capsule (Plate 4). The parenchymal vasculature was not visible in spleen.



Plate 4: Sagittal plane In-vitro sonogram of spleen: 1. Parenchyma 2. Capsule 3. Base of container

Real-time ultrasonographic Study:

Acoustic Window: Spleen was seen from 6th to 11th intercostal spaces (ICS) of left abdomen in the animals of G1. Dorsal margin of spleen was visible in ventral third of 6th, 7th, and 8th intercostal spaces; mid portion of 9th and 10th intercostal spaces and in upper third of 11th intercostal space. Ventral

margin of spleen ended in ventral third of 6th, 7th, 8th and 9th intercostal spaces mid portion of 10th and upper portion of 11th ICS. In the animals of G2 and G3 Spleen was seen from 8th to 12th intercostal spaces with dorsal and ventral margins following almost the same pattern of G1 animals (Plate 5).

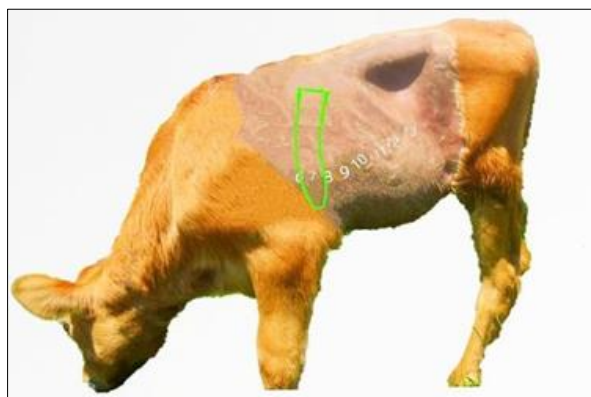


Plate 5: Acoustic widow for ultrasonographic Spleen scan in calves

Echogenicity patterns: Splenic parenchyma was uniformly echogenic in nature. Capsule appeared as a fine hyperechoic line on the diaphragmatic surface but was not appreciated

over its visceral surface (Plate 6). Vasculature appeared as oval or circular in cross section and elongated in longitudinal section as hypoechoic structures in parenchyma.



Plate 6: A: Cross section, B: Longitudinal view Real Time Ultrasonogram of Spleen at 8th & 9th ICS 1. Capsule, 2. Parenchyma 3.dorsal sac of Rumen 4.Splenic tail 5. Abdomen muscles and fascia Cr. Cranial Cd. Caudal

Location: The spleen was visualized in the animals of all groups on the left side of abdomen. It was situated between the costal part of the abdominal wall and the rumen or the reticulum. The dorsal part of the spleen was in contact with the diaphragm and superimposed by the lung. The dorsal and ventral visible margins of the spleen ran from cranioventral to caudodorsal directions. In the animals of G3, spleen extended ventrally far enough to contact the liver.

Biometry: Spleen was of the largest size 7.03 ± 0.56 cm at 6th, 12.81 ± 0.51 cm at 7th and 13.73 ± 0.516 cm at 8th ICS in the animals of G1, G2 and G3 respectively. Smallest size of spleen was observed at 4th and 12th ICS in the animals of G1 and at 5th and 12th ICS in the animals of G2 and G3 respectively. Thickness of the spleen increased with increase in age of calves from G1 to G2 with measurements in thickness varying respectively in these groups as 1.52 ± 0.12

cms, 2.21 ± 0.09 cms and 2.15 ± 0.23 cms. Thickest portions of spleen were observed in the animals of all groups from 9th to 12th intercostal spaces and thinnest in 7th ICS. The length of spleen progressively increased from G1 to animals (Table 1). Splenic size and thickness of G1 animals were statistically significant ($P \leq 0.05$) from those of G2 and G3 animals, however the values in G2 and G3 animals were none significantly ($P > 0.05$) different between them. Splenic length values of animals in different groups were significantly ($P < 0.05$) different from one another (Table 1).

Observations with regard to organ location and echogenicity pattern obtained from cadaveric study, organ bath study and RTU had fair resemblance. Overall echogenicity observed in RTU was lesser than that obtained in organ bath study. Vasculature was fairly visible in RTU as compared to organ bath study. Splenic capsule at visceral surface adjacent to rumen or reticulum as not well appreciated in RTU.

Table 1: Mean \pm SE (cm) of calf spleen echobiometry “upper values” in animals of different groups

Groups	Parameters		
	Spleen Size	Spleen length	Spleen thickness
G1	7.03 ± 0.56^a (5.57-8.50)	9.65 ± 0.36^a (8.7-10.6)	1.52 ± 0.12^a (1.2-1.8)
G2	12.81 ± 0.51^b (11.48-14.14)	13.83 ± 0.46^b (12.6-15.0)	2.21 ± 0.09^b (1.9-2.4)
G3	13.73 ± 0.516^b (12.4-15.0)	15.35 ± 0.33^c (14.5-16.2)	2.15 ± 0.23^b (1.5-2.7)

Values with same Superscript ^{a, b, c} represents statistical non significance.
Range in parentheses as 95% CI

Discussion Cadaveric Study

Spleen is the largest lymphoid organ having own haemopoietic activity (Aspinall *et al.*, 2015) [2]. Spleen in neonatal calves, remain within rib cage and does not extend to last rib (Raymond and Stanlay, 2010) [12], however in adult cattle spleen extends up to dorsal end of last two ribs to costo-condral junction of 7th and 8th rib (Bhardwaj *et al.*, 2013) [3]. Dorsal extremity is under upper ends of last 2 ribs and ventral extremity is opposite to 7th - 8th rib about hands breadth above costo-condral junction. Parietal surface is related to diaphragm while visceral surface to rumen and reticulum (Sisson, 1975) [14]. In the present study similar observations were noted and the spleen was seen located caudodorsally distal to T₁₁ to T₁₃ vertebrae spreading medially to last three

to two vertebra (11th to 12th ICS). The apex was cranioventrally located medial to distal third of 6th to 7th ICS in the animals of group 1 and 7th ICS in group3 animals thereby remaining within rib cage in all the groups of calves.

In adult cattle spleen is related to dorsocranial surface of rumen and cranial surface of reticulum below and diaphragm above (Bhardwaj *et al.*, 2013) [3]. Diaphragmatic surface is applied to the diaphragm (Budras and Habel, 2003) [6]. However, in the present study spleen base was mostly associated with dorsal rumen sac especially in neonatal and preruminant calves. The calves of groups 3 were having nearly similar position.

Splenic length of adult cattle is 40-50cm while as its width is 12-15 cm. Its middle portion is thickest and having thickness

of 2-3 cm (Sisson, 1975) [14]. In the present study spleen was 8-9 cm in length and its width at broadest part was 4.5- 5 cm in the animals of G1 while as average length of spleen in G2 & G3 animals was recorded 12 and 15.5 cm and width at broadest part was 5 and 7 cm respectively. The variation is obvious because of the age difference.

Organ bath Study

During this study 12 MHz linear transducer was found appropriate for both longitudinal and transverse views of spleen in comparison to the 3.5 MHz curvilinear transducer used in other study (Imran *et al.*, 2011) [11] for having optimum scan. Parenchyma showed isoechoic granular pattern with an enveloping thick hyperechoic capsule. The parenchymal vasculature was not visible in spleen. Clear mirror-image artifact, reported by Imran (2010) [10] in his study was not observed during scanning of spleen. Proper alignment of transducer along the different parts of the organ scanned in water bath could have prevented it to occur during our study.

Real time ultrasonographic study of normal spleen

Spleen is a storage area for red blood cells and filters blood through a sinusoidal system (Rowen *et al.*, 2009) [13]. It has additional functions in lymphopoiesis, antibody production, and 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 in response to systemic states (Douglas *et al.*, 2010) [8]. Systemic inflammations cause a regular and fairly predictable pattern of response in the spleen. 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 hemorrhage. There is no published data, characteristic signs of splenic disease and specific methods for examination of this organ in calves especially at different stages of their life. It cannot be palpated externally and there is no specific laboratory test for determining splenic disease. Ultrasonography is an ideal, non-invasive method to examine the bovine spleen (Braun and Sicher 2006) [5]. All the compartments of the ruminant stomach develop from a primordium and relative sizes of the four compartments change with age (Sisson, 1975) [14]. Thus topography of abdominal organs adjacent to spleen (rumen, abomasum, and intestine) is different in calves as compared to cows, so ultrasonographic reference of spleen in cow is useless for neonatal calves. Spleen of neonate calves in lateral recumbent position has been evaluated (Chit sazi, *et al.*, 2012) [7], but not of calve at different stages of life. This study to describe the ultrasonographic appearance, size and location of the spleen and associated blood vessel in healthy calves of different age groups is thus of utmost importance.

Spleen was seen from 6th to 11th intercostal spaces (ICS) of left abdomen in G1 animals and 8th to 12th ICS in G2 and G3 calves respectively and its dorsal as well as ventral margins running cranioventral to caudoventral. Capsule appeared as a fine hyperechoic line on the diaphragmatic surface but was not appreciated over its visceral surface usually due to the superimposition of rumen. Vasculature appeared as oval or circular in cross section and elongated in longitudinal section

as hypoechoic structures in parenchyma. The spleen was situated between the costal part of the abdominal wall and the rumen or the reticulum. The dorsal part of the spleen was in contact with the diaphragm and superimposed by the lung. The views were almost similar to the study conducted by Chit Sazi *et al.* (2012) [7] and Braun and Kruger (2013) [4] however the acoustic window in former ranged from 7th to 12th ICS in neonatal calves and in latter study it was 5th to 12th ICS for calves ranging from neonatal to above 3 months of age.

Largest spleen size was recorded at 6th, 7th and 8th ICS in the animals of G1, G2 and G3 while as smallest size of spleen was observed at 4th and 12th ICS in the animals of G1 and at 5th and 12th ICS in the animals of G2 and G3 respectively. Thickest portion of spleen was recorded from 9th to 12th intercostal spaces and thinnest in 7th ICS in all groups. The length of spleen progressively increased from G1 to G3 animals. These findings were in agreement with Braun and Kruger (2013) [4] however, the findings were in disagreement with Chit Sazi *et al.*, (2012) [7] who reported smallest size of spleen recorded in the 8th intercostal space and largest in the 11th intercostal space in neonatal calves.

Conclusion

The size, location, position and sonographic features of the spleen in calves show considerable variation according to their age and differ from those of adult animals. Ultrasonography is an excellent diagnostic tool for evaluating the spleen in calves.

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