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Anatomical and Ultrasonographical Studies on the Spleen of Red Fox (*Vulpes Vulpes*) from Western Egyptian Desert

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ABSTRACT

This study provides preliminary data about anatomical and ultrasonographical features of the spleen in clinically normal red fox, using the distinct orientation of the splenic artery. Six red foxes of both sexes with an average weight of 4-6kg and age range 2-3years were achieved from the Western Egyptian Desert. Trans-abdominal splenic ultrasonography was performed on all foxes with the dorsal recumbent position. The foxes were euthanized by an intravenous overdose of Thiopental sodium. Anatomical dissection was performed on two sacrificed animals while the other four animals were used to describe the arterial architectures (n=two foxes) and angiography (n=two foxes). The splenic parenchyma appeared slightly hyperechoic to isoechoic relative to the adjacent liver. It was hypoechoic to the surrounding fat. The spleen of the red fox had a falciform outline and was located in the left cranial abdominal cavity, beneath the last two ribs just cranial to the left kidney. The red fox's spleen was unique in its relation to the left crus of the diaphragm and the absence of renosplenic ligament. The splenic artery was the last branch of the celiac trunk and was subdivided into two main vessels, dorsal and ventral. These branches entered the spleen through two splenic hila at both extremities. Splenic arteriography revealed an internal link between these vessels but lacked an external connection.

Key words: Angiography, Red Fox, Spleen, Splenic arteries, Ultrasonography

INTRODUCTION

The red fox belongs to Family: *Canidae*, Genus: *Vulpes* and Species: *Vulpes vulpes*. It is the largest of true foxes and one of the most widely distributed members of order Carnivora (Lindblad-Toh et al. 2005). This wild animal is a wide-ranging species ranging from arctic tundra to deserts and urban areas. It occurs across Europe, Africa, Asia and North America (Hoffmann and Sillerozubiri 2021).

Egypt is home to 93 species of mammals of which 20 species of the Carnivora. There are four species of foxes in Egyptian deserts and Nile Valley. The red fox is very widespread in Egypt (Richard 2003). Moreover, several Egyptian zoological gardens have hundreds of red foxes. Therefore, this study can provide valuable preliminary data about the red fox's spleen. These data may help veterinary surgeons and radiologists in diagnosing various splenic disorders in this species.

The red fox is an important fur porter and more upraised on farms than any other wild fur-bearing animal. Their pelts are applied for trimmings, scarfs, muffs, jackets and coats. Moreover, the red fox helps in the control of populations of small rodents as it has a characteristic manner of hunting mice (Carter et al. 2012).

The spleen is considered the largest reticuloendothelial hematopoietic tissue in animals. It is an elongated and relatively flat organ located in the cranial left abdominal region and attached to the stomach via small blood vessels (Maronezi et al. 2015; Jaji et al. 2019).

Splenic diseases are not uncommon in species taxonomically close to the red fox (Spangler and Culbertson 1992). Several splenic diseases like congenital disorders, splenomegaly, hyperplastic lymphoid nodules, hematomas, twisting, rupture; tumors, cysts and others have been recorded in animals (Spangler and Culbertson 1992; Nazem et al. 2019).

Many animals with splenic diseases present with vague clinical signs therefore, splenic ultrasonography is very essential to assess the splenic status (Mattoon and Nyland 2014). Trans-abdominal splenic ultrasonography is one of the paramount techniques used for evaluating the spleen in small animal practice. The size, shape, echogenicity, and echotexture of the spleen should be evaluated (Mattoon and Nyland 2014). The abnormalities,

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like splenomegaly along with parenchymatic alterations in echotexture and echogenicity, could be identified by ultrasonography (Maronezi et al. 2017). Describing the vascular anatomy of the spleen is paramount for angiologists along with veterinary surgeons (Fouad et al. 2018a). Angiography offers complimentary data about vascular architecture (El-Bably and Abouelela 2021).

According to the available veterinary literature, there is only one study describing the histomorphology of the spleen in the fox (*Vulpes bengalensis*) (Firdous and Maya 2013). Therefore, the comparative anatomy of the red fox's spleen with other animals' spleens is lacking. This work illustrates - for the first time - the normal anatomical and ultrasonography details of the spleen of a red fox with special reference to angioarchitures of the splenic artery.

MATERIALS AND METHODS

Ethical Approval

This study was approved by the Institutional Animal Care and Use Committee at the Faculty of Veterinary Medicine, Cairo University, Egypt (Vet. CU.12/10/2021/343). Also, all international regulations for animal care and use were followed up. All efforts were done to minimize the stress and pain of the foxes.

Animals

Six red foxes of both sexes (Three males and three females) and 2-3years old were enrolled in this study. Their weight ranged between 4-6kg. They were obtained from the Western Egyptian Desert. All foxes were clinically normal.

Ultrasonography Examination

After fasting for 8 hours but drinking water was available, the red foxes were sedated by intramuscular injection of Xylazine HCl (Xylaject 2%®, ADWIA, Egypt) at a dose of 1mg/kg body weight. The abdominal hair was clipped and ultrasound coupling gel was applied. B-scan trans-abdominal ultrasound was done using a 5 to 10-MHz linear transducer (Hitachi Aloka F31, Tokyo, Japan). Ultrasound examination was performed in a semi-dark room where the foxes were manually restrained in the dorsal recumbent position.

The splenic ultrasonography was started at the craniodorsal extremity of the spleen along the cranial border; the transducer was moved along the abdominal wall from the left cranial abdomen to the left caudal abdomen until the entire cranial border of the spleen had been imaged. The ultrasonography examinations were carried out by the same operator (MHH).

Anatomical Dissection of the Red Fox's Spleen

The sedated foxes were sacrificed just after ultrasonography for anatomical dissection and angiography. They were sacrificed by an overdose of Thiopental sodium 5% solution given intravenously (60mg/kg). The sacrificed foxes were divided randomly into three groups (two foxes each) for anatomical dissection, vascular architectures and angiography of the splenic artery.

Two sacrificed red foxes were used for descriptive anatomy by further dissection of the specimens and isolating the spleen from its surrounding organs.

The Vascular Architecture of the Splenic Artery of the Red Fox

Another two foxes were cannulated through the abdominal aorta and infused by prepared red latex neoprene according to previous studies (El-Bably and Abouelela 2021; Abdelnaby et al. 2021), for showing the splenic artery and its distribution.

The last two sacrificed foxes were used for splenic arteriography to describe the vascular anatomy of the splenic artery and its major branches within the splenic parenchyma by using an X-ray machine and contrast medium (Fischer, Stuttgart, Germany). The red foxes were inoculated by a mixture of turpentine oil plus lead oxide into the celiac trunk. The radiographic position was a medial view for the spleen and radiographic setting factors included 40kVp and A.

RESULTS

Normal Ultrasonography Features of the Red Fox's Spleen

The landmarks for detection of the spleen included the stomach that was located cranial and medial to the spleen, the descending colon that was located dorsal, medial and caudal to the body of the spleen and the left kidney that was located dorsal, medial and caudal to the spleen (Fig. 1). The red fox's spleen was imaged along the left cranial and ventral abdominal wall that paralleled the greater curvature of the stomach within the greater omentum.

The spleen appeared as an elongated, solid organ. It was neither thick nor long, measuring 0.4-0.5cm in thickness at the apex and 0.5-0.6cm at the base, 1.5-2cm in width at the apex and 2.5-3cm at the base and 7-8cm in length. The red fox's spleen was seen in the near field. When the transducer was positioned in a long axis relative to the fox (craniocaudal), the spleen appeared as a short-axis triangular shape.

The splenic parenchyma was slightly hyperechoic to isoechoic relative to the adjacent liver. It was hypoechoic to the surrounding fat. Also, the red fox's spleen was slightly hyperechoic relative to the adjacent left kidney. The spleen had an outer hyperechoic thin capsule (Fig. 1).

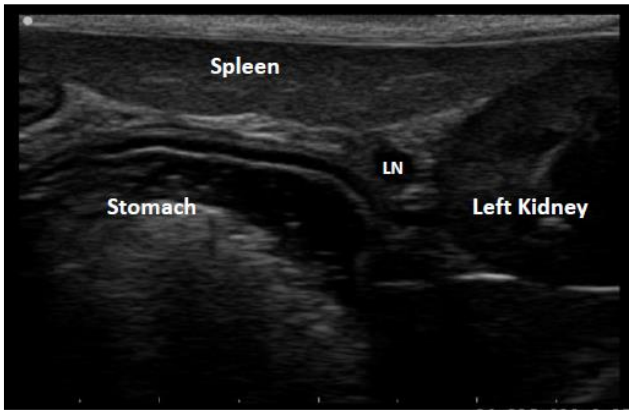

Fig. 1: Long-axis sagittal ultrasonogram of the normal spleen in a red fox. Notice the stomach, left kidney and the splenic lymph node (LN).

The spleen normally showed homogeneous echotexture, but when using a high-frequency linear array transducer, the spleen showed coarser architecture and a more heterogeneous appearance. The splenic lymph node appeared as a circular hypoechoic structure as shown in (Fig. 1). Also, the splenic blood vessels could be seen at the splenic hilum.

Normal Anatomical Features of the Red Fox's Spleen

The spleen of a red fox was reddish-brown and had a falciform shape (Fig. 2 and 3). It had a narrow dorsal apex and wide ventral base and took a curved position dorsally in the left cranial abdominal region (Fig. 2). It was further down the last two ribs and extended just caudal to the last rib (13$^{th.}$rib) by 1-1.5cm. It concealed the greater curvature of the stomach (Fig. 3B) and related dorsally to the left crus of the diaphragm while caudally it was just cranial to the left kidney (Fig. 2). The spleen was attached to the stomach by the gastrosplenic ligament while the renosplenic ligament was absent.

The red fox's spleen had two surfaces, lateral costal smooth surface (Fig. 3A) and medial surface that had the hilus. The splenic hilus is divided into two portions, proximal and distal. These portions were not connected externally but arterial communication was seen internally.

The splenic length was 7.1-8.4cm while the width at its apex was 1.8-2.2cm and at the base was 2.8-3.2cm (Fig. 3A). The splenic thickness measured 0.5-0.6cm at the apex and 0.6-0.7cm at the base.

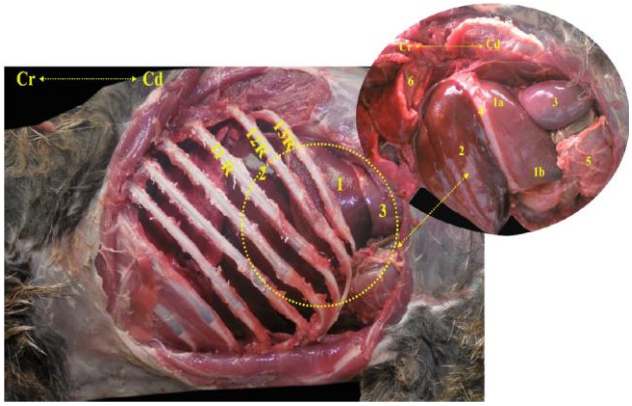

Fig. 2: Photograph showing the lateral abdominal wall of the red fox after removing the abdominal muscles with focusing on the spleen in situ.

The Architecture of the Splenic Artery of the Red Fox

The splenic artery in the red fox was the third division of the celiac artery (Fig. 4A) and could be considered its direct continuation. It is released after the hepatic and gastric arteries (Fig. 4A/13&14, respectively). The splenic artery passed for a short distance then bifurcated into two dominant arteries, dorsal and ventral (Fig. 4B/16&17, respectively). The dorsal dominant one ramified four apical splenic twigs (Fig. 4A/18) on the apex of the spleen then, continued as short gastric arteries (two small vessels) (Fig. 4A/19) that supply the greater curvature of the stomach at its left extremity near to the esophagus. The ventral dominant vessel (Fig. 4A and B/17) emanated four small pancreatic arterioles to the pancreas (Fig. 4B/21) then lengthened till reached the base of the spleen where it subdivided into, proximal (Fig. 4B/22) and distal branches (Fig. 4B/23). The proximal branch of the ventral dominant artery ramified on the spleen by three basal splenic arterioles (Fig. 4B/24) subsequently continued cranially as left gastroepiploic to arborize on the greater curvature of the stomach by eleven branches (Fig. 4B/25). The distal branch of the ventral dominant one was responsible for nourishing the parenchyma of the splenic base with another basal splenic arteriole (Fig. 5C/24) then continued as an omental branch (Fig. 4B, 5A&B/26).

Splenic angiography revealed intra-parenchymal communication between dorsal and ventral dominant branches through an anastomosis between the ramified twigs of both (Fig. 5B and D/27).

DISCUSSION

Studies on anatomy and ultrasonography of wild animals like a red fox (*Vulpes vulpes*) are very scanty in the veterinarian database. Therefore, this study was planned to describe the normal anatomy and ultrasonography of the spleen in red fox with special emphasis on the vascular anatomy of the splenic artery. The obtained results of the current study are very important for veterinary surgeons and radiologists.

Regarding the splenic ultrasonography, unlike in deep-chested dogs, there was no need to use the intercostal left-sided approach through the 11$^{th.}$and 12$^{th.}$ intercostal spaces to image the dorsal extremity of the spleen in the red foxes. This difference could be attributed to the small size of the red foxes.

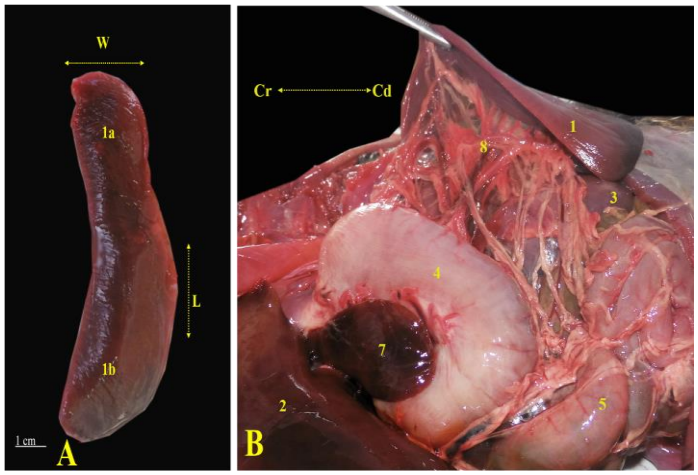

Fig. 3: A) Photograph showing the costal surface of red fox's spleen and its dimensions. **B)** Photograph showing relation of red fox's spleen in situ (reflected spleen).

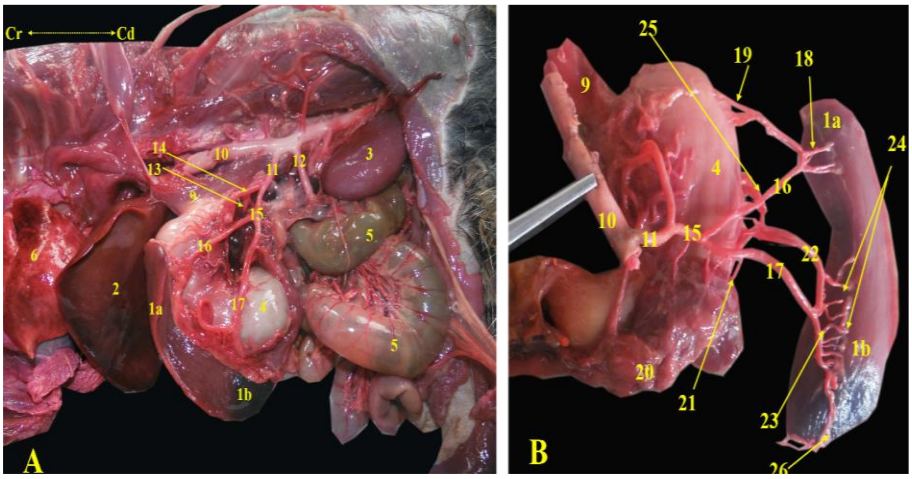

Fig. 4: A) Photograph showing the origin of the splenic artery with reflected spleen in situ. **B)** Photograph showing the splenic artery after reflection of the stomach.

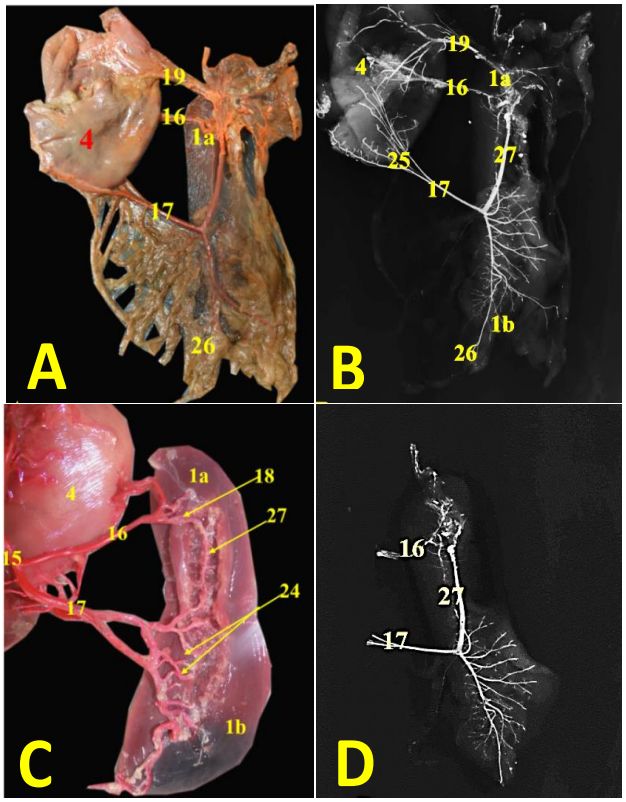

Fig. 5: (A) Photograph showing main branch of the splenic artery in relation to the stomach. **(B)** Splenic arteriogram showing the main branch of splenic artery in relation to the stomach. (C) Photograph showing the communicating branches of the splenic artery. **(D)** Splenic arteriogram showing the communicating branches of the splenic artery.

Legend of Figures

1: spleen, a- apex, b- base, 2: left lateral lobe of the liver, 3: left kidney, 4: greater curvature of the stomach, 5: intestines, 6: lung, 7: papillary lobe of the liver, 8: gastrosplenic ligament, 9: esophagus, 10: abdominal aorta, 11: celiac trunk, 12: cranial mesenteric artery, 13: hepatic artery, 14: gastric artery, 15: splenic artery, 16: dorsal dominant splenic artery, 17: ventral dominant splenic artery, 18: four apical splenic twigs, 19: short gastric arteries, 20: pancreas, 21: pancreatic branches, 22: the proximal branch of the ventral dominant artery, 23: the distal branch of the ventral dominant artery, 24: basal splenic arteriole, 25: left gastroepiploic artery, 26: omental branch, 27: communicated branch between dorsal and ventral dominant splenic artery.

Because the spleen was only attached by the gastrosplenic ligament of the greater omentum, its position in red foxes can be variable. Moreover, changes in the size, shape, echogenicity and echotexture of the spleen may be a normal response and maybe sonographically indistinguishable from a significant pathologic process. Therefore, a normal splenic scan does not mean an absence of diseases. Similar findings were reported before in puppies (Hwang 2020).

The spleen normally showed homogeneous echotexture, but when using a high-frequency linear array transducer, the spleen showed coarser architecture and a more heterogeneous appearance. This is in a line with the results of a previous study on puppies (Hwang 2020).

The obtained results are essential for the comparative anatomy between the red fox and other animals. For example, the obtained results revealed that the red fox's spleen is reddish-brown similar to that of Bengal fox (Firdous and Maya 2013). In addition, the red fox's spleen has a narrow dorsal apex and wide ventral base. In contrast in the buffalo calf, the spleen has a bright purple colour, with a dorsal broad border and narrow rounded ventral end (Noor and Maher 2018).

As regards the splenic length, the average splenic length in red fox is 7.1-8.4cm, whereas it is 34.5-38.5cm in buffalo calf, 22.5-31.5cm in camel, 12.5-14.5cm in sheep and 6.5-7.5cm in goat (Noor and Maher 2018). While it reaches 16±0.1cm in Bengal fox (Firdous and Maya 2013). Regarding the dog, the average splenic length is highly variable according to the different breeds (Maronezi et al. 2017). Moreover, this study revealed that the thickness of the red fox's spleen is 0.4-0.5cm at the apex and 0.5-0.6cm at the base. While in a dog, the maximum thickness of the normal spleen is 1.7cm (Maronezi et al. 2017).

Regarding the anatomical site of the spleen, the red fox's spleen is placed over the greater curvature of the stomach that is similar to the dog's spleen (Dyce et al. 2010). Moreover, the spleen of the red fox contacts with the cranial pole of the left kidney while it is expanded to the caudal pole in camels (Jaji et al. 2019). The red fox spleen is located in the left cranial quadrant of the abdominal cavity. Similar findings have been recorded in Bengal fox (Firdous and Maya 2013) and dogs (Sutila et al 2017).

The red fox's spleen is falciform in outline, while it is semilunar in camel (Jaji et al. 2019), elongated elliptical in buffalo calf, triangular in sheep (Noor and Maher 2018),

comma-shaped triangular in the Bangladesh horse (Alam et al. 2005) and tongue-shaped in Bengal fox (Firdous and Maya 2013).

Interestingly, the present results revealed that the spleen of the red fox is only attached to the stomach by gastrosplenic ligament as in donkey (Fouad et al. 2018b) In contrast, the camel's spleen is attached to the left kidney, panaceas and rumen through reno-splenic, splenopancreatic and gastrosplenic ligaments, respectively, (Jaji et al. 2019; Noor and Maher 2018). Also, the horse spleen has the renosplenic ligament (Fozfilho et al. 2013). In line with previous studies in dogs (Dyce et al. 2010; Barr and Gaschon 2011) and rabbits (Dimitrov 2012), the renosplenic ligament is not verified in our study.

Our results revealed a single main splenic artery in the red fox that bifurcated into dorsal and ventral dominant branches. However, in a dog, it splits into dorsal, middle and ventral stem vessels (Misk and Hifiny 1978). These results disagree with the results obtained from the camels and buffalo calves where two separate branches and three primary branches are ensured, respectively (Noor and Maher 2018). Moreover, the splenic artery in the red fox is subsequently divided about 3-4cm before reaching the hilum. This is in agreement with the results obtained from cattle (Takcl 2009) and camels (Maina et al. 2014). While in the dog it is divided about 5-6cm (Misk and Hifiny 1978). On the other hand, the splenic artery is divided just after its entrance into the splenic hilum in both sheep and horse (Fozfilho et al. 2013; Noor and Maher 2018).

In red fox, the dominant splenic branches enter the hila through the dorsal and ventral extremity of the spleen, whereas in the dog it enters the hilum through dorsal, middle and ventral segments (Misk and Hifiny 1978). On the other hand, the splenic hilum is present at the cranial border below the proximal extremity in both sheep and buffaloes (Noor and Maher 2018).

The dorsal dominant splenic artery in red fox ramifies on the apex of the spleen and the ventral one is responsible for nourishing the base. These results disagree with the results obtained from camels and horses, where the ventral extremity is vascularized by the first branch of the splenic artery and the dorsal end of the spleen is irrigated by the second branch (Fozfilho et al. 2013; Maina et al. 2014; Noor and Maher 2018).

Interestingly, angiography revealed intra-splenic communication between the arterial radicles of the main arteries within the splenic tissue of the red fox. Also, an arterial anastomosis either between the branches of the same artery or between branches of the primary splenic arteries was reported in cattle (Takcl 2009). While no anastomosis within splenic parenchyma was recorded in camels (Noor and Maher 2018).

The main limitations of this study were the relatively small number of foxes and lack of splenic histopathology thus; the results should be considered as preliminary data and interpreted with caution. Further studies on larger numbers of red foxes and various ages are recommended. Moreover, further studies on other organs of the red foxes are recommended to reach a complete normal anatomic and sonographic atlas about this wild animal to interpret any abnormalities in these organs.

Conclusión

The red fox has characteristic anatomic and ultrasonographic features. This study establishes preliminary data about these features and reference values of the spleen in a clinically normal red fox that will help diagnose red foxes with splenic diseases.

Author's Contribution

AMI and SMD: designed the research work, performed the anatomical representation of the spleen and described its arterial manner. AMA and MHH: performed the ultrasonography and angiography. All authors revised the manuscript and approved the last version of the manuscript.

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