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Ultrasonographic and gross anatomical characterization of superficial lymph nodes in donkey (*Equus Asinus*)

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ABSTRACT

This study aimed to describe the ultrasonographic (dimensions and echogenicity), anatomical and histological features of superficial lymph nodes (LN) (mandibular, superficial cervical, subiliac, scrotal, and supramammary) in donkeys. Twenty-four donkeys (3-6 years old and 120-180 kg BW) were examined ultrasonographically for characterization of LN which were dissected and processed for histological examination. Data showed that mandibular LN, located between mandibular rami along each side of omohyoid muscles, ultrasonographically appeared as long-lobulated structures with clear anechoic lobules and hyperechoic interlobular trabeculae. Superficial cervical LN, located at 5 cm dorsal to the shoulder joint at the caudal end of the jugular vein, ultrasonographically appeared as a quadrilateral to oval multi-lobulated structure with hypoechoic lobules interspersed with fine interlobular echogenic trabeculae and delineated by a thick distinct echogenic capsule. Subiliac LN, located at the midway between the patella and coxal tuber, ultrasonographically appeared as an oval wholly anechoic structure with a fine central hypoechoic septum delineated by a thick hyperechoic capsule. Scrotal LN, located at the level of the superficial inguinal ring either cranial or caudal to the spermatic cord, ultrasonographically appeared as a small ovoid to rounded shaped structure with an entire anechoic content. Supramammary LN, localized on the lateral border of the udder base at the superficial inguinal ring, ultrasonographically appeared as a rectangular to ovoid structure with an entire anechoic content. In conclusion, the present data set a preliminary descriptive reference for normal ultrasonographic imaging as well as the topographical anatomy for adult donkeys' superficial LNs.

1. INTRODUCTION

Donkey (*Equus asinus*), a domesticated member of Equidae, are used as working animals, especially in developed countries, for draught and riding (Davis et al. 2016). Donkeys have unique characteristics that make them of physiological and medical interest to scientists, veterinarians, and equine enthusiasts (White 2013). The incidence of lymphatic system disorders and malignancy is continuously increasing in donkeys (Adedokun et al., 2020 and Davis et al., 2016). This makes the diagnostic imaging and characterization of LN in donkeys crucial.

Lymph nodes, the body's immunological organ, are found throughout the body (Blum and Pabst 2006). Inflammation, viral invasion, metastatic malignant tumors of served organs, and primary lymph node malignancies will all result in alterations in LN size, shape, and architecture (Roozendaal et al. 2008). As a result, lymph node abnormalities may signal the probability of organ diseases in the region (Mountain and Dresler 1997). Furthermore, during clinical illness therapies, appropriately interpreting the nature of lymph nodes not only helps in disease diagnosis but may also guide treatment plan selection and considerably enhance patient prognosis (Kayani et al. 2011). Furthermore, the qualitative and quantitative evaluation of lymph nodes is critical in the treatment of malignant tumours (Pantuck et al. 2003). Ultrasound examination technology is currently a widespread imaging approach for evaluating the lymphatic system and may give a foundation for the examination of several lymphatic problems (Białek and Jakubowski 2017, Hayashi et al. 2018 and Ogassavara et al. 2016).

For more than two decades, ultrasonography has been used as a highly accurate and cost-effective diagnostic tool for superficial lymph node assessment (Białek and Jakubowski 2017, Esen 2006 and Zhang et al. 2009). Several ultrasound parameters can be estimated including LN size, margins and echo pattern (echotexture) (Nyman et al. 2005). To the author's knowledge, no literature has discussed the

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ultrasonographic and macroscopic features of donkeys' superficial lymph nodes. Therefore, the present study aimed to characterize superficial LN in donkeys through topographic anatomical dissection and Ultrasonographic imaging which could assist in diagnosing lymphatic disorders in equines.

2. MATERIAL AND METHODS

2.1. Ethics Statement

The present study was conducted and approved by the Ethical Committee for Institutional Animal Use and Care of the College of Veterinary Medicine, Menoufia University Egypt.

2.2. Animals

A total of twenty-four (12 males and 12 females) donkeys (*Equius asinus*) aged between 3-6 years old, weighing 120-180 kg, owned by private farmers at Toukh city, Qalubia Governorate, Egypt, were used in the present study. Before enrollment, all donkeys were subjected to a complete physical, and clinical (rectal temperature, heart, and respiratory rates, lung sound, and mucous membranes) examination to ensure that they were clinically healthy (Mendoza et al. 2018).

2.3. B-Mode ultrasonographic examination

2.3.1. Area preparation and examination

All ultrasound examinations were performed on none-sedated quietly restrained donkeys, in standing position, in a quiet environment with low light and controlled temperature using a portable ultrasound machine (Chison ECO3-Expert, Medical-EXPO, China) equipped with a multi-frequency linear probe (Janvier et al., 2016). All superficial LN: mandibular, superficial cervical (prescapular), Subiliac (prefemoral), and superficial inguinal (scrotal LN in males, and supramammary LN in females) were examined per cutaneous. All ultrasound scanning was settled at a frequency of 7.5 MHz, depth of 5 cm, acoustic power of 80%, and gain of 150 dB. The area of examination was shaved and cleaned with alcohol, and a liberal amount of coupling ultrasound gel was applied before each transcutaneous ultrasonography.

2.3.2. Probe maneuvering

Ultrasonographic examination of mandibular LN was achieved by placing the probe in the intermandibular space parallel to each ramus at its medial aspect forming a V shape area of scanning. The scanning surface of the probe was directed cranially upward (Fig. 1A). The superficial cervical LN was discriminated ultrasonographically by placing the probe just cranial to the anterior border of the scapula perpendicular to the jugular groove before the thoracic inlet and directing the scanning surface of the probe slightly caudal with light pressure (Fig. 1B).

The Subiliac LN was examined mid-way between the coxal tuber and patella in the prefemoral groove (cranial edge of thigh). We used the point of the hip for approaching by drawing a longitudinal line extending cranially from the hip to cut a line extending ventrally from the middle of the tuber coxae, both lines cutting area was the level of the subiliac LN. The scanning surface of the probe was slightly directed caudal with light pressure (Fig. 1C). For the ultrasonographic approach of the scrotal LN, the probe was placed on each side of the penis at the level of the superficial inguinal ring and either cranial or caudal to the spermatic cord after offsetting the scrotum to the opposite side. The scanning surface of the probe was directed dorsally and slightly lateral with light pressure (Fig. 1D). For ultrasonographic examination of the supra-mammary LN, the probe was placed on the lateral border of the base of the mammary gland after offsetting the mammary gland to the opposite side. The scanning surface of the probe was directed dorsally and slightly lateral with light pressure (Fig. 1E).

2.4. Image Analysis

For image analysis, the ultrasonographic images were frozen and the length, width, and length/width ratio were measured using the built-in calibre system (Mattoon and Nyland, 2002). All images were then exported to coma puter and analyzed with Image J software (Image J, NACL Co. Ltd., Tokyo, Japan) to calculate the mean pixel value of each lymph node cortex and the adjacent tissues (Kandiel and El Shafey 2017).

2.5. Surgical anatomy of lymph nodes using black India ink staining

Six randomly selected donkeys (three males and three females) were used for studying the surgical anatomy of LN using black India ink staining to validate the ultrasound observations. A mixture of India ink solution (3-5 ml) and bovine serum (1:1) was used for accurate observation of the examined LN (Sayed-Ahmed, 2009). The animals were injected subcutaneously within the left side at the area known as drained by the examined LN, at least 10-15 cm away from the node to avoid over-staining of the node. For mandibular LN, the injection was carried subcutaneously into the mentum and cheek regions. For the superficial cervical LN, the injection was carried at the middle cervical region and just caudal to scapula over the triceps brachii muscle. For the subiliac LN, the injection was carried at the level of the last three ribs. For scrotal LN, the injection was carried within the wall of scrotum on both sides of the scrotal raphe. For supramammary LN, the injection was carried out bilateral subcutaneous in mammary gland. Twenty-four hours' post-injection, the donkeys were intravenously premedicated using 1.1mg/kg xylazine *(Xylaject, Adwia Co., Cairo, Egypt)* for intravenous induction of general anesthesia by using 10 mg/kg sodium thiopental 5% (Anapental, Sigma Co., *Cairo, Egypt*) (Alsobayil et al., 2019). The donkeys were then sacrificed by bleeding through the common carotid artery and were perfused using an ordinary preservative mixture (10% formalin, 2% phenol, and 1% glycerin) through the common carotid artery. Forty-eight hours later, the cadavers were dissected, and the topographical anatomy of each examined LN was evaluated.

2.6. Histological investigation of examined LN

After topographical investigation, LN were dissected out from the cadavers and preserved in 10% neutral buffered formalin. The LN samples were dehydrated in ascending grades of ethanol, cleared in xylene, and impregnated in melted paraffin wax. Thin sections (5-7 µm thick) were cut, mounted on egg albumin-glycerin coated glass slides, stained with hematoxylin and eosin (H&E) stain, and examined under a light microscope (Elmore, 2006).

2.7. Statistical analysis

Data were expressed as mean (± SEM). All statistical analysis was conducted using IBM® SPSS® Statistics Ver. 20 program (IBM Corporation, 2009, New York, USA). The correlation between LN ultrasound and gross measures was calculated using Pearson's correlation coefficient. P value was set at 0.05 to demark the statistical significance.

4. RESULTS

3.1. Ultrasonographic and topographical anatomy of superficial lymph nodes in donkey

Table 1: Ultrasound and topographic measures (length, width, length/width ratio) of superficial lymph nodes in donkeys.

Lymph node (LN)		Length (cm)				Width (cm)				Length/width ratio	
		Mean ± SE		Range		Mean ± SE		Range			
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Mandibular LN	US (n=12)	6.55 ± 0.19[a]	6.57 ± 0.18[a]	5.71 - 7.61	5.64 - 7.51	1.32 ± 0.05[a]	1.31 ± 0.05[a]	1.10 - 1.70	1.08 - 1.69	5.04 ± 0.22[a]	5.09 ± 0.22[a]
	TA (n=3)	6.70 ± 0.84[a]	6.67 ± 0.76[a]	5.3-8.2	5.5-8.1	1.77 ± 0.15[a]	1.77 ± 0.23[a]	1.5-2.0	1.4-2.2	3.77 ± 0.17[a]	3.79 ± 0.07[a]
Superficial cervical LN	US (n=12)	7.92 ± 0.28[a]	7.96 ± 0.26[a]	6.40 - 9.70	6.87 - 9.80	3.99 ± 0.09[a]	3.98 ± 0.11[a]	3.50 - 4.44	3.36 - 4.41	1.99 ± 0.08[a]	2.02 ± 0.09[a]
	TA (n=3)	8.40 ± 1.04[a]	8.33 ± 1.01[a]	6.7-10.3	6.6-10.1	4.37 ± 0.54[a]	4.40 ± 0.61[a]	3.6-5.4	3.4-5.5	1.92 ± 0.041[a]	1.90 ± 0.03[a]
Subiliac LN	US (n=12)	3.47 ± 0.09[a]	3.47 ± 0.10[a]	2.93 - 4.11	2.94 - 4.20	1.09 ± 0.07[a]	1.06 ± 0.08[a]	0.83 - 1.45	0.43 - 1.45	3.32 ± 0.20[a]	3.69 ± 0.58[a]
	TA (n=3)	3.67 ± 0.32[a]	3.70 ± 0.12[a]	3.1-2.4	3.5-3.9	1.40 ± 0.10[a]	1.43 ± 0.15[a]	1.3-1.6	1.2-1.7	2.67 ± 0.38[a]	2.64 ± 0.30[a]
Scrotal LN	US (n=12)	2.50 ± 0.12		2.12 - 3.10		0.82 ± 0.04		0.66 - 0.98		3.11 ± 0.19	
	TA (n=3)	2.70 ± 0.32		2.1-3.2		0.88 ± 0.13		0.66-1.1		3.09 ± 0.091	
Supra-mammary LN	US (n=12)		4.10 ± 0.19		3.10 - 5.00		2.16 ± 0.07		1.92 - 2.60		1.92 ± 0.11
	TA (n=3)		4.21 ± 0.54		3.2-5.03		2.37 ± 0.23		2.0-2.8		1.86 ± 0.39

US: ultrasound. TA: topographic anatomy

Variable with different superscript letters in the same row are significantly different at P≤0.05.

3.1.1. Mandibular lymph nodes

Ultrasound and topographical measures including length, width and length/width ratio of superficial LN in both males and females was recorded in (Table 1).

Ultrasonographically, mandibular LN in donkeys appeared as a homogenous lobulated structure with clear anechoic lobules, hyperechoic interlobular loose connective tissue trabeculae and delineated by regular a thick distinct echogenic capsule (Fig. 2A). Ultrasonographically, mandibular LN length was 6.55 ± 0.19 and 6.57 ± 0.18 and its width was 1.32 ± 0.05 and 1.31 ± 0.05 in male and female, respectively. Anatomically, mandibular LN is arranged in two elongated groups between the two rami of mandible within the intermandibular space along each side of the omohyoid muscles. The two groups were in apposition in front of the insertion of omohyoid muscles, diverged caudally in the form of a V shape structure. Each mandibular LN was formed of aggregation of about 40-70 small nodes that were strongly attached by fascial sheath forming one lymphatic mass. The anatomical landmarks for mandibular LN were caudal third of intermandibular space and the vascular groove of both mandibular rami. For approaching, at the caudal third of intermandibular space and just rostral to vascular groove of mandible, the mandibular LN was related deeply to the rostral part of omohyoid and sternohyoid muscles and sublingual salivary gland (Fig. 2B).

3.1.2. Superficial cervical (prescapular) lymph node

Superficial cervical LN was ultrasonographically scanned at a depth of 1.5-2.0 cm beneath the skin line. It appeared as a homogenous quadrilateral to oval-shaped multi-lobulated structure delineated by a regular thick distinct echogenic capsule. It had hypoechogenic lobules interspersed with fine interlobular echogenic loose connective tissue trabeculae (Fig. 2C). Ultrasonographically, superficial cervical LN length was 7.92 ± 0.28 and 7.96 ± 0.26 and its width was 3.99 ± 0.09 and 3.98 ± 0.11 in male and female, respectively. Anatomically, superficial cervical LN is composed of an aggregation of small nodes that grossly gave the nodes a large lobulated and granulated mass. Anatomical landmarks of the superficial cervical LN were the shoulder joint, the caudal end of the jugular groove, and the divisions of brachiocephalic muscle (Cleidomastoid and cleidobrachialis muscles). For approaching the superficial cervical LN, it was localized 5 cm dorsal to the shoulder joint at the cranial border of the subclavius muscle and medial to the Cleidomastoid and Omotransversarius muscles and lateral to the omohyoid and scalenus muscles as well as the caudal end of the jugular vein (Fig. 2D).

3.1.3. Subiliac (prefemoral) lymph nodes

The Subiliac LN was scanned by B-mode ultrasound at 1.0-1.5 cm below the skin line. It appeared as homogenous oval shape structure delineated by a regular distinct thick hyperechoic capsule. It looked like a wholly anechoic structure with a fine central hypoechoic septum (Fig. 2E). Ultrasonographically, subiliac LN length was 3.47 ± 0.09 and 3.47 ± 0.10 and its width was 1.09 ± 0.07 and 1.06 ± 0.08 in male and female, respectively. Anatomically, subiliac LN consisted of an aggregation of small lymph nodes (n= 10-15) connected forming an elongated lymphatic mass. The anatomical landmarks were the coxal tuber, patella and point of hip, and the cranial border of the tensor fascia latae muscle. For approaching the subiliac LN, it was located midway between patella and coxal tuber on craniomedial side to tensor fascia late muscle (cranial edge of thigh) and lateral to the aponeurosis of the external and internal abdominal oblique muscles. Also, the point of hip was used for approaching LN by drawing a longitudinal line extending cranially from hip to cut a line extending ventrally from the middle of tuber coxae. Both lines intersected at the level of the subiliac lymph node (Fig. 2F).

3.1.4. Superficial inguinal lymph nodes (scrotal in male and supra-mammary in female)

In the male, superficial inguinal (scrotal) LN was scanned ultrasonographically at 2.0-2.5 cm beneath the skin line. It appeared as a small oval to round shape structure with the entire homogenous anechoic contents, delineated by regular hyperechoic capsule and embedded in a large amount of echogenic fat tissue of the inguinal region (Fig. 3A). Ultrasonographically, scrotal LN length was 2.50 ± 0.12 and its width was 0.82 ± 0.04. Scrotal LN appeared as a small aggregation of LNs on both sides of the penis at the level of the superficial inguinal ring and either cranial or caudal to the spermatic cord. The anatomical landmarks were the penis, scrotum, and spermatic cord. The approaching was performed on both sides of penis along the spermatic cord till the superficial inguinal ring where LN appeared either cranial or caudal to the spermatic cord and close to the superficial inguinal ring and embedded in large amount of fat tissue of the inguinal region (Fig. 3B).

In female, superficial inguinal (supramammary) LN was scanned ultrasonographically at 0.5-1.0 cm beneath the skin line. It appeared as homogenous rectangular to ovoid structure with an entire anechoic content, delineated by a regular fine hyperechoic capsule and embedded in a large amount of echogenic fat tissue of the inguinal region (Fig. 3C). Ultrasonographically, supramammary LN length was 4.10 ± 0.19 and its width was 2.16 ± 0.07. Anatomically, supramammary LNs were relatively larger and with a higher number of small nodes than that observed in scrotal. The LNs were localized on the lateral border of the base of mammary gland at the superficial ring of the inguinal canal (Fig. 3D). The anatomical landmarks were the lateral border of the base of the mammary gland, superficial inguinal ring, and the highest part of medial aspect of the thigh where LNS can be manually palpated.

3.2. Correlation between superficial LN ultrasound and gross measures

Analysis of the correlation between ultrasound and topographic anatomy measures of donkey superficial LN (Fig. 4 A and B) verified a significant ($P<0.001$) positive correlation between the measured length and width of the examined LN ($R^2 = 0.7468$ and $R^2 = 0.8713$, respectively). Echo-pattern analysis showed a significant ($P< 0.05$) variation in the mean gray value between LN, with the mandibular LN was the lowest and the scrotal LN was the highest in the pixel values (Table 2). On the other hand, all LNs were comparatively less echogenic than the adjacent tissues, with the echogenicity reduction varying significantly among LN compared to the surrounding tissues. Prescapular LN was extremely hypoechogenic (reduction rate 63.35±2.22%) than surroundings.

Table 2: Ultrasonographic echo-pattern characteristics of superficial lymph nodes in donkeys

Lymph node	Pixel value		Pixels value reduction (%)
	Cortical tissue	Adjacent tissue	
Mandibular LN	31.17±1.47^{c}	69.59±1.47	54.89±2.53^{ab}
(Range)	(21.12-38.12)	(59.11-75.49)	(39.60-70.60)
Prescapular LN	38.94±1.51^{b}	108.10±3.56	63.35±2.22^{a}
(Range)	(32.56-46.38)	(86.14-128.67)	(47.32-72.58)
Prefemoral LN	33.67±1.28^{bc}	71.26±1.05	52.61±2.05^{b}
(Range)	(24.47-39.01)	(62.61-75.49)	(39.60-63.98)
Scrotal LN	51.55±1.41^{a}	114.20±2.45	53.16±3.04^{b}
(Range)	(42.99-58.14)	(99.51-128.67)	(34.50-67.72)
Supramammary LN	47.82±2.15^{a}	104.20±4.16	54.71±1.35^{ab}
(Range)	(35.20-56.56)	(82.44-120.30)	(46.79-60.61)

Data was expressed as mean ± SEM (n=24). Means with different letters within the same column were significantly different at $p< 0.05$

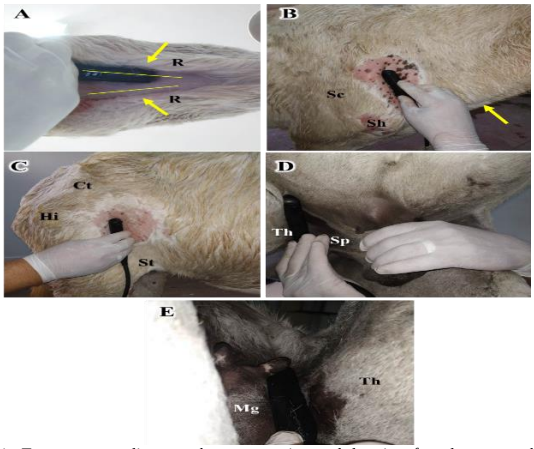

Fig.1. Transcutaneous linear probe maneuvering and location for ultrasonographic scanning of superficial lymph nodes in donkey. (A) Mandibular lymph nodes; showing mandibular ramus (R), the level of the vascular groove (arrow). (B) Superficial cervical (prescapular) lymph nodes; showing shoulder joint (Sh), scapula (Sc) and the jugular groove (arrow). (C) Subiliac (prefemoral) lymph nodes; showing hip joint (Hi), the coxeal tuber (Ct) and stifle joint (St). (D) Superficial inguinal (scrotal) lymph node; showing medial aspect of thigh (Th) and spermatic cord within intact neck of the scrotum (Sp). (E) Superficial inguinal (mammary) lymph node; showing medial aspect of thigh (Th) and mammary glands intact (Mg).

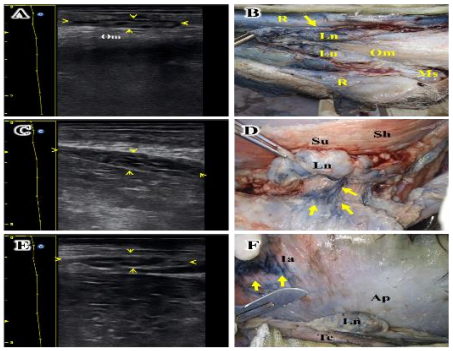

Fig. 2. Ultrasonographic scanning of mandibular lymph nodes (demarcated by arrow heads) (Panel A) and the topographical anatomy of examined region (Panel B) showed mandibular lymph nodes (Ln), mandibular ramus (R), vascular groove (arrow), omohyoid muscle (Om) and mandibular salivary gland (Ms). Ultrasonographic scanning of superficial cervical (prescapular) lymph nodes (demarcated by arrow heads) (Panel C) and the topographical anatomy of examined region (Panel D) showed superficial cervical lymph (Ln), shoulder joint (Sh), afferent vessels (arrows), subclavius muscle (Su). Ultrasonographic scanning of Subiliac (prefemoral) lymph nodes (demarcated by arrow heads) (Panel E) and the topographical anatomy of examined region (Panel F) showed subiliac lymph node (Ln), common aponeurosis of external and internal oblique muscles (Ap), afferent vessels (arrows), fleshy part of internal abdominal oblique muscle muscle (Ia) and tensor fascia lata muscle (Te).

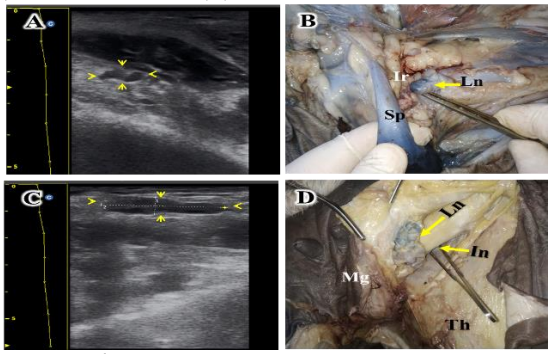

Fig. 4. Analysis of the correlation between ultrasound and topographic anatomy measures of the superficial lymph nodes in donkey.

3.3. Histological architecture of donkey lymph nodes

All dissected LNs were surrounded by adipose tissue and covered by fibrous connective tissue capsule from which several trabeculae extended into the substance of LNs, subdividing the outer region of the cortex into incomplete compartments. The afferent lymph vessels pierced the capsule on the convex surface of LN and empty their lymph into the subcapsular sinus, deep into the capsule. This sinus was continuous with the cortical sinuses (para-trabecular sinuses) that parallel the trabeculae and deliver the lymph into the medullary sinuses. In all examined LNs, the subcapsular, cortical and medullary sinuses were filled with the Indian ink specially the first two sinuses (Fig. 5A-E).

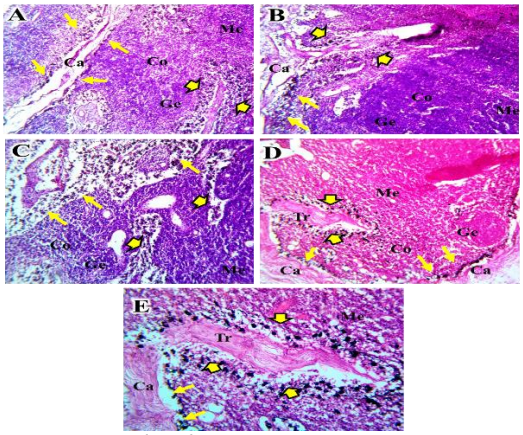

Fig.5. Photomicrograph of superficial lymph nodes in donkey showing the capsule of the lymph node (Ca), the subcapsular sinus filled with injected Indian ink (arrows), cortical sinuses filled with Indian ink (thick arrows) cortex (Co), germinal center (Ge) and medulla (Me) in mandibular lymph node (A), superficial cervical (prescapular) lymph node (B), subiliac (prefemoral) lymph node (C), Superficial inguinal (scrotal) lymph node (D) and Superficial inguinal (mammary) lymph node (E).

4. DISCUSSION

Attempts to establish a standardized reporting system have been made for several organs and body systems in donkey including the digestive, urogenital and musculoskeletal system (Sikandar, 2020)**,** but no literature have discussed the topographic anatomy and ultrasonography of donkey superficial LNs. The current study aimed to characterize the ultrasonographic, anatomical, and histological features of superficial LNs in donkeys. Such experience has a clinical significance in the diagnosis and follows up several conditions encountered with lymphatic disorders such as lymphadenitis and malignancy. Moreover, the offered measurements represented a preliminary guideline of the expected sizes of superficial LNs in the healthy donkey.

In the current study, B-mode ultrasonography appears to be a useful tool to scan the entire structure of superficial LNs in donkeys and create a clear border definition with sufficient contrast to the surrounding tissue. This experience has been used in humans for lymphatic scanning (Abdelgawad et al., 2020). In veterinary practice, ultrasonography was used as a highly accurate and cost-effective diagnostic tool for LN scanning and diagnosing of lymphatic disorders in dogs (Citi et al., 2020; Nyman and O'Brien, 2007; Pugh, 1994), cats (Schreurs et al., 2008; Spattini et al., 2003), and mice (Walk et al., 2014)**.**

All LNs examined in our study exhibit the same homogeneity, uniform contour lining, oval to elongated shape and comparatively less echogenicity than the adjacent (Schreurs et al., 2008). Similarly, ultrasound scanning of normal LNs in humans revealed an elongated to oval shape structure with hyperechoic hilum and clearly hypoechoic cortex (Ogassavara et al., 2016). In the present study, the described anatomical features of the mandibular LN were nearly similar to that of horse where the mandibular LN formed from 10 cm of conglomerate mass with nearly 70 – 150 small nodes and situated ventral to the tongue and rostral or caudal to the level of vascular groove of the mandible (Dyce et al., 2009; Getty and Sisson, 1975). On the other hand, mandibular LN in ruminants is a single large LN, rather than groups of small nodes (Budras et al., 2003). Moreover, (Mansour et al., 2017) stated that mandibular LNs in ruminants are 1–3 LNs that are located rostral and lateral to the mandibular salivary glands behind the caudal angle of the mandible.

Similar to previous reports in the horse, the superficial cervical LNs were localized nearly 5 cm dorsal to the shoulder joint at the cranial border of the subclavius muscle and were viewed as an aggregation of several small nodes that grossly gave the nodes a large lobulated or granulated mass (Dyce et al., 2009; Getty and Sisson, 1975). Nevertheless, the superficial cervical LNs were not easy to be palpated since the LNs were surrounded by large amounts of connective and fat tissue. In horse, the superficial cervical LN is arranged in a long chain consists of many small nodes that cross the deep surface of the brachiocephalic muscle and are embedded in fat tissue. Palpation should be directed to drawing the nodes forward, away from the subclavius (Flood, 1996). In the contrary, the superficial cervical LNs is easily palpated in ox compared to equines (König and Liebich, 2020). Walk et al. (2014) added that, cervical lymph node of mice evaluation by clinical ultrasound is a non-invasive procedure used in diagnosing nodal status.

In the present study, the subiliac LNs formed from an elongated lymphatic mass (3.67±0.32 cm in male and 3.70 ± 0.12 cm in female) of small LNs (n= 10-15) located midway between patella and coxeal tuber on craniomedial side to tensor fascia lata muscle. This observation matched the finding that has been reported in horses previously (Dyce et al., 2009; Getty and Sisson, 1975). In contrary, the subiliac LNs in ruminants appeared as a single palpable larger mass (8-10 cm in length) and located 12 -15 cm dorsal to the patella near the cranial border of the tensor fasciae lata (Dyce et al., 2009; Getty and Sisson, 1975).

The superficial inguinal (scrotal) LN described in male donkey here agreed with that reported previously in the horse (Budras et al., 2003). The scrotal LN interposed between the prepuce and scrotum, and trunk, and drains lymph from the external reproductive organs and ventral abdominal wall (Flood, 1996). In ox, the scrotal LNs is formed of varied number LNs and lies in a mass of fat around the neck of the scrotum and partially covered by retractor penis muscle (Mansour et al., 2017)**.** The superficial inguinal (mammary) LN in she-donkey was relatively larger with a higher number of small nodes than that observed in males. This result paralleled with (Damian et al., 2009), who reported that the mammary LN of the mare is situated between the abdominal wall and the base of the udder at the superficial inguinal ring with 10-14 cm in length. In cow, the mammary LN comprises two LNs lie caudally on the base of the udder and can be palpated between the thighs, about 6 cm from the skin, at the caudal attachment of the udder and may be associated with smaller intramammary nodes (Dyce et al., 2009; Getty and Sisson, 1975). In accordance with the current study, superficial inguinal lymph nodes in ewe and lactating cattle could be examined by using a 7.5 MHz linear transducer (Bradley et al., 2001 and Hussein et al., 2015). The same authors concluded that the superficial inguinal lymph nodes ultrasonography is a fast field test for confirming the diagnosis of subclinical mastitis. The superficial inguinal lymph nodes in subclinical mastitis could be easily recognized and appeared as oval-shaped structure with thin echogenic capsules, sharply demarcated from the adjacent tissue and entire anechoic or hypoechoic parenchyma and linear echogenic structure at the center. Moreover, ultrasonographic parameters including length, depth and area of the superficial inguinal lymph nodes were significantly increased in the udder diseased groups.

Analysis of the correlation between ultrasound and topographic anatomy measures of donkey superficial LN verified a significant positive correlation between the measured length and width of the examined LN which means that ultrasonography is a reliable method for the examination of superficial lymph nodes in the donkey.

5. CONCLUSIONS

In conclusion, the present data provide baseline information on normal ultrasonographic imaging as well as the topographical anatomy of the superficial LNs of adult donkeys. Moreover, our study confirms that the ultrasonography is a costless, non-invasive reliable diagnostic tool for evaluating the status of superficial LN in donkeys as well as horses.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR'S CONTRIBUTION

Conceptualization by Khalil AH and Abd Al-Galil AS. Data curation by Khalil AH, Ahmed S-A and Abd Al-Galil AS. Formal analysis by Khalil AH and Kandiel MM. The methodology by Khalil AH, Abd Al-Galil AS, and Ahmed S-A. Writing – original draft by Khalil AH and Ahmed S-A. Writing - review and editing by Khalil AH, Abd Al-Galil AS and Kandiel MM.

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