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Ultrasonographical, Morphological and Histological Studies on Jugular Vein Conduit of Cattle and Buffalo

Basma Barakat[1], Hatem B. Husseiny[1], Ahmed A. Kassab[1], Ahmed I. Abo-Ahmed[1], Ahmed H. Khalil[2*], Anwar A. El-shafey[1]

[1]Departments of Veterinary Anatomy and Embryology, Faculty of Veterinary Medicine, Benha University, Benha, Egypt.
[2]Departments of Veterinary Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Benha University, Benha, Egypt.

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ABSTRACT

Xenografting using bovine jugular vein (BJV) (Vv.jugulars) valved conduit is recently introduced for reconstruction of different cardiovascular disorders. Commercially prepared conduit needs a standard technique for selection of suitable animal and jugular vein segment with ideal characters of Valved conduit. The present study was carried out on 10 adult healthy animals (5 cattle (Bos-Taurus) and 5 buffaloes (*Bubalus bubalis*) and 10 cadaveric BJV specimens collected from slaughtered healthy adult animals (5 cattle and 5 buffaloes). The study aimed to establish a standard method for choosing the suitable animal and segment of JV that would be used for post-slaughtering collection of conduits. Ultrasonographically, morphological and histological characteristics of JV in cattle and buffaloes were also studied and compared. Ultrasonography of JV was performed along its length from the mandible till the thoracic inlet. The assessed ultrasonographic JV features included; vein lumen width (VLW), JV wall thickness (VWT), distance between valves and venous wall gray scale analysis. Ultrasonographically, venous tricuspid valve appeared in both planes (sagittal and transverse) of the 4th quarter segment in both animals. The VLW; significantly increase in cattle than buffaloes. The VWT; significantly increased in buffaloes than in cattle. Morphologically, CJV has less thick and less tough wall; and wider lumen when compared with BJV. Histologically, JV wall is 3-layered; tunica intima (inside), tunica media and tunica adventitia (outside) and the wall thickness of BJV are thicker than CJV. In conclusion, the 4th quarter of CJV is the most appropriate segment advised for post-mortem collection of JV conduits. Ultrasonography is an essential, prerequisite technique for choosing the suitable animal and the perfect segment of JV conduits.

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Introduction

Valved conduit tissue grafts are frequently used for reconstruction of wide varieties of cardiovascular disorders including right ventricular outflow tract (RVOT) reconstruction, pulmonary valve replacement (PVR) and Peripheral Artery Disease (PAD) (Flores and Yu, 2017). Nevertheless, despite rigorous clinical and experimental studies, the perfect valved conduit couldn't be established. The accessibility of appropriate pulmonary homografts is restricted, particularly for urgent procedures. Frequently used xenografts, including valves built from bovine pericardium and porcine aortic valves; but necessitate physical manipulation and deficient to stability and durability (Schwarzwald and Jenni, 2009). Natural heterologous valved conduits are frequently used as a substitute to other xenografts and homografts, however mid-term consequences may be complicated by severe perigraft scarring, excessive intimal peel formation and supravalvular stenosis (Okamura *et al*., 2019). B- Mode ultrasonography has been introduced as a simply performed reliable and non-invasive diagnostic tool for assessment of the physiologic reference values and a wide range of pathological conditions for jugular veins in human (Okamura *et al*., 2019) and healthy horses (Pasolini *et al*., 2019). To the best of the author knowledge, a comparison between jugular vein dimensions and morphological characteristics between cattle and buffaloes have not been stated so far. For this, the aim of this study was to determine the significant points of comparison of jugular vein anatomy via ultrasonography, morphological and histological investigation. Moreover, the study aimed to establish a standard method for choosing the suitable animal and the perfect segment of jugular vein that would be most appropriate for post-slaughtering collection of jugular veins conduits at lumen diameter within and up to 22 mm.

*Corresponding author: Ahmed H. Khalil
E-mail address: ahmed.khalil@fvtm.bu.edu.eg

Materials and methods

Experimental animals

The present study was carried out on ten adult healthy animals (5 cattle and 5 buffaloes) and ten cadaveric specimens of jugular vein collected from slaughtered healthy adult animals (5 cattle and 5 buffaloes).

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Ultrasonography

Ultrasonographical examinations were performed in a standing position within stanchion according to Pasolini *et al*. (2019). Unsedated animal was quietly restrained by lateral deviation of the head to other side. After clipping of the hair and application of coupling ultrasound gel, ultrasonography of Jugular vein was accomplished by using a portable ultrasound machine (Chison ECO3-Expert, Medical-EXPO, China) with an adapted multi-frequency linear probe set at 8 MHz. Firstly, the normal right jugular vein was defined then a thumb compression was applied at the thoracic inlet to keep the jugular lumen engorged and the entire vein was scanned from the head systematically towards the thoracic inlet, to rule out any clinically imperceptible changes, which would have led to the exclusion of the vein from the study. Ultrasonographic examination was performed in both longitudinal and transverse planes on each segment between the mandibular angle and the thoracic inlet. The assessed ultrasonographic jugular vein features included vein lumen width (VLW), the wall thickness (VWT) and the distance between valves. Ultrasonographic scan of each segment was done as a new start, therefore releasing the thumb pressure on the jugular vein for at least 30 seconds; avoid sliding movements of the probe between segment, and a coupling gel application were repeated. The best available acquisitions in both cross and the sagittal planes at each segment were freezed and analyzed for image analysis (Pasolini *et al*., 2019). Ultrasonographically, quantitative measurement of vein wall echogenicity was achieved by image brightness analysis (on gray scale units from 0 (black) to 255 (white)) by using dedicated software Image J (Image J, NACL Co. Ltd., Tokyo, Japan) to obtain the mean gray value of the analyzed ultrasonographic image (Grani *et al*., 2015; Mostafa *et al*., 2016).

Ethics statement

The study procedures were conducted in compliance with the guidelines for the care and use of animals at the College of Veterinary Medicine, Benha University, Egypt. The research protocol was approved by the Ethical Committee for Institutional Animal Use and Care of the College of Veterinary Medicine, Benha University (BUFVTM 15-09-2020).

Tissue sampling

Right jugular veins were obtained from a local slaughterhouse in Toukh city immediately after slaughtering process. Ensuring that slaughtering process occurring under sanitary conditions, then all samples were prepared by rinsing with phosphate-buffered saline (PBS) 7.4 to remove any blood and body fluids. Jugular vein samples were transported from slaughterhouse to the laboratory in ice box and just after arriving to the laboratory; tissue samples were dissected to eliminate any adherences then prepared for gross anatomical study and histological examination.

Macroscopic examination

Jugular vein samples were carefully inspected and imaged using a digital camera (Nikon, Tokyo, Japan). The pictures were processed with the Adobe Photoshop CS software (Adobe, SanJose, CA, USA) (Gauvin *et al*., 2013).

Histological examination

Specimens of jugular vein were fixed for 72 hours in 10% neutral buffered formalin, then were dehydrated in ascending grades of alcohol, cleared in xylene, and embedded in paraffin. Paraffin blocks were cut into sections of 5 µm thick. The sections were stained with hematoxylin and eosin for general structure (Bancroft and Gamble, 2007).

Statistical Analysis

The SPSS software version 22 was used to analyze the data. Analysis of variance was used to examine the data (Anova). Means and standard error means were used to present the data. $P<0.05$ was used to declare the data to be different.

Results

Normal jugular vein was identified in ultrasonography as a collapsed structure with thin anechoic sections of blood flow without any blocking to blood flow within the vein lumen. Normal distended cattle jugular vein (C-JV) (Fig.1) and buffalo jugular vein (B-JV) (Fig. 2) appeared in sagittal plane ultrasonography as anechoic tubular structure outlined with a thin, hyperechoic wall and discrete borders of the endothelial surface (Fig. 5C). Transverse plane ultrasonography of unvalved area of CJV (Fig. 3A, B) and un-valved area of BJV (Fig. 3G, 3H) showed round to oval anechoic structure with smooth

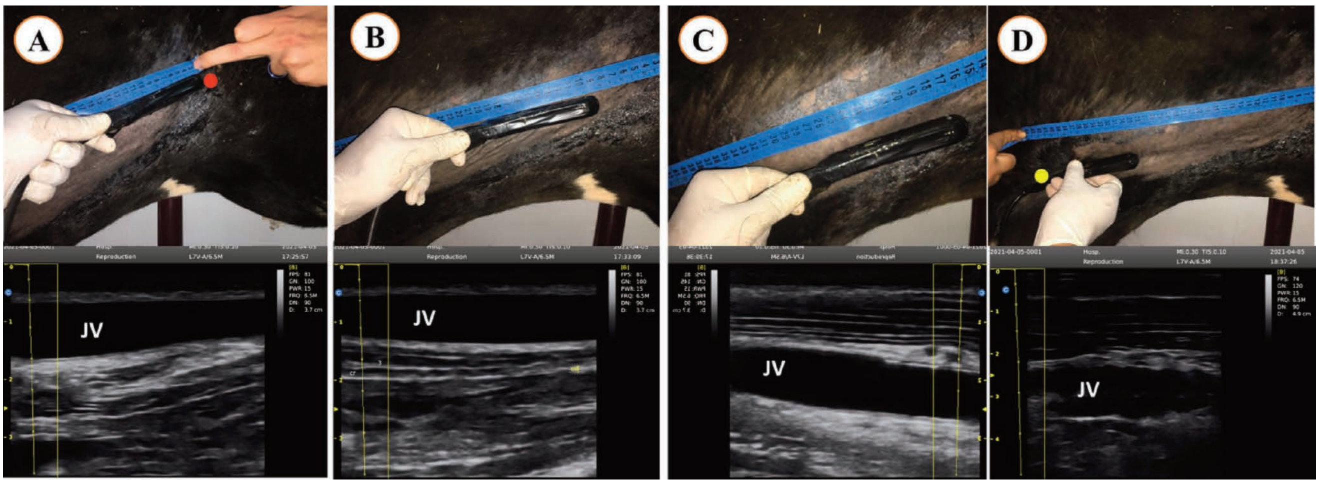

Fig. 1. Surface Ultrasound scanning showing probe maneuvering and the crossponding ultrasonographic image of each quarter of the cow jugular vein (JV) (the first quarter (A), the second quarter (B), the third quarter (C) and the fourth quarter (D)). The beginning point of scanning at the mandibular angle (A-red point), the end point of scanning at the thoracic inlet (D-yellow point).

echogenic lining. Venous tricuspid valve was recognized ultrasonographically in both sagittal and transverse planes of the fourth quarter segment in both animals. The number of discriminated valves was two to three in each vein. Transverse plane ultrasonography of the tricuspid valve in cattle (Fig. 3D, 3E) and buffaloe (Fig. 3J, 3K)

Jugular veins showed a three hypo echogenic leaflets attached to the endothelial lining of the vein and extended as cup shape structure toward the venous lumen. Sagittal plane ultrasonography of the venous tricuspid valve in cattle (Fig. 4A, 4B) and buffaloe (Fig. 4C, 4D) jugular veins revealed a couple of echogenic bands extended from the endothelial lining toward the venous lumen. Findings of jugular vein ultrasonographic features in both cattle and buffalo were presented in (Table-1). CVLW and buffalo vein lumen width BVLW revealed a significant increase ($P<0.05$) in 2nd, 3rd and 4th quarter than in the first one. CVLW showed significant increase ($P<0.05$) than BVLW in all quarters. Venous lumen width in both animals increased gradually along the vein length from cranial (head side) to caudal (heart side). CVWT and buffalo vein wall

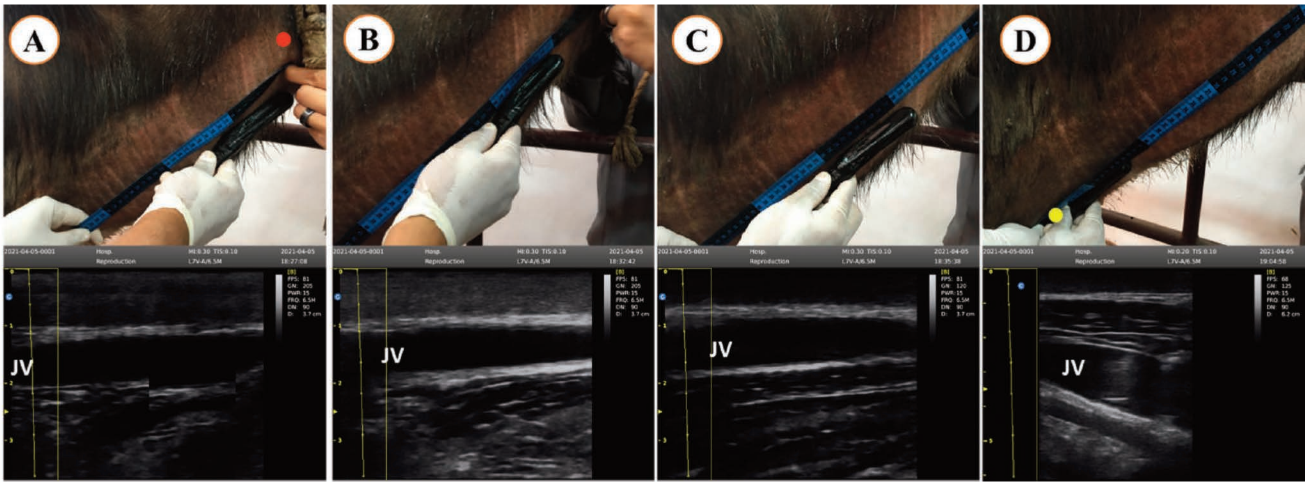

Fig. 2. Surface Ultrasound scanning showing probe maneuvering and the crossponding ultrasonographic image of each quarter of the buffalo jugular vein (JV) (the first quarter (A), the second quarter (B), the third quarter (C) and the fourth quarter (D)). The beginning point of scanning at the mandibular angle (A-red point), the end point of scanning at the thoracic inlet (D-yellow point).

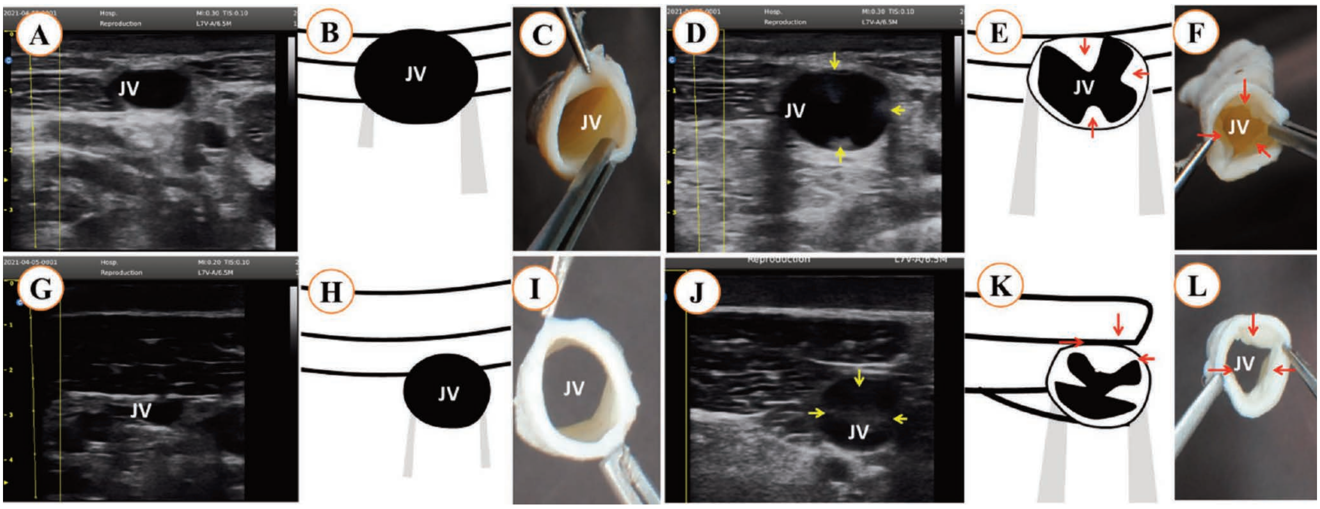

Fig. 3. Unvalved area of Cow jugular vein; Transverse Plane Ultrasonography (A), Schematic presentation of ultrasonography (B) and gross view (C). Valved area of Cow jugular vein; Transverse Plane Ultrasonography (D), Schematic presentation of ultrasonography (E) and gross view (F). Unvalved area of Buffaloe jugular vein; Transverse Plane Ultrasonography (G), Schematic presentation of ultrasonography (H) and gross view (I). Valved area of Buffaloe jugular vein; Transverse Plane Ultrasonography (J), Schematic presentation of ultrasonography (K) and gross view (L). Jugular Vein (JV), Valve (arrows).

Table 1. Ultrasonographic features of jugular vein ultrasonography (first quarter, second quarter, third quarter and fourth quarter) in cattle and buffaloes.

Feature		First Quarter	Second Quarter	Third Quarter	Fourth Quarter
Vein Lumen Width (VLW) (mm)	Cattle	18.5 ± 0.84^{a1}	22.4 ± 0.55^{a2}	22.8 ± 1.14^{a2}	23.2 ± 1.3^{a2}
	Buffaloes	15.01 ± 0.84^{b1}	17.1 ± 0.89^{b1}	17.6 ± 0.84^{b1}	18.1 ± 0.55^{b1}
Wall Thickness (WT) (mm)	Cattle	1.76 ± 0.11^{a1}	1.78 ± 0.11^{a1}	1.9 ± 0.16^{a1}	1.9 ± 0.18^{a1}
	Buffaloes	2.04 ± 0.09^{b1}	1.96 ± 0.11^{b1}	2.02 ± 0.084^{b1}	2.3 ± 0.07^{b1}
Wall Gray Scale Analysis (WGSA) (unit)	Cattle	56.2 ± 0.84^{a1}	57 ± 1.87^{a1}	58.8 ± 2.17^{a1}	59.4 ± 2.30^{a1}
	Buffaloes	114.8 ± 1.92^{b1}	116.2 ± 2.17^{b1}	117 ± 1.87^{b1}	119.2 ± 4.92^{b1}
Distance between valves (mm)	Cattle	-	-	-	3.46 ± 0.35^{a}
	Buffaloes	-	-	-	5.54 ± 0.45^{b}

The mean within the same row having different superscripts (numbers) and the mean within the same column having different superscripts (letters) are significantly different at level ($p < 0.05$). (The measuring unit is millimeter).

thickness BVWT revealed non-significant difference between the 4 quarters. BVWT showed significant increase ($P<0.05$) than CVWT in all quarters.

The distance between valves showed significant ($P<0.05$) decrease in cattle than in buffalo. Vein wall gray scale analysis showed significant decrease ($P<0.05$) in cattle than in buffalo when compared in all quarters (Fig. 5A, 5B). Morphological macroscopic examination of jugular vein in cattle (Fig. 3C, 3F) and buffalo (Fig. 3I, 3L) showed that; the jugular vein was found directly under the skin in the 1st, 2nd and 3rd quarters of the neck and deeper in the end of 3rd quarter and 4th quarter of the neck in the jugular between furrow brachiocephalic muscle dorsally and sterno-cephalic muscle ventrally pass superficial to omo-hyoideus muscle. The shape of lumen is oval or round and the diameter of C- JV sections along its length is increased from cranial to caudal so it resembles conical shape. Comparing cattle and buffalo in diameter found that buffalo diameter is narrower than cattle. The wall of CJV is softer and less thick than BJV. In addition, the terminal segment of JV contains natural tricuspid valve which consists of three very thin movable leaflets; one leaflet usually larger than the other two leaflets. Histologically, in both species, the jugular vein wall was consisted of three layers (from inside to outside), which included, tunica intima, tunica media and tunica adventitia from inside to outside in both animals. Tunica intima represented by endothelial lining (single layer of simple squamous epithelium) and underneath a few elastic fibers which flapped nearly the lumen. Tunica media is very thin layer consist of 2-3 layers of smooth muscle fiber. Tunica media is poorly demarcated from the tunica adventitia. The third layer, tunica Adventitia, is evident bulky layer and contains connective tissue fibers as collagen and elastic fibers. The only difference found between 2 animals as the wall thickness of BJV is thicker than CJV (Fig. 6).

Discussion

The limited accessibility of homografts has obligate surgeons to search for substitute tissue valved conduits for reconstruction of wide varieties of cardiovascular disorder including RVOT reconstruction, PVR and PAD, especially for urgent interferences (Flores and Yu, 2017). The jugular vein conduits (Contegra) is a glutaraldeyde-treated xenograft which recently introduced as commercial alternative with a promising result incomparable to that of homografts (Breymann *et al*., 2002). To the best of the author knowledge, assessments of jugular vein diameters have not been stated so far in cattle and buffaloe in a comparison. This study intended to determine the significant points of comparison of jugular vein anatomy via ultrasonography, morphological and histological examinations. Ultrasonographic examination of normal

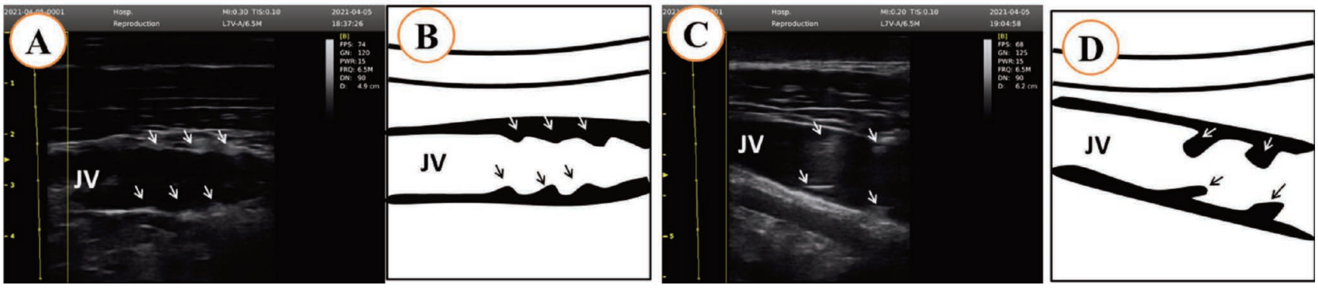

Fig. 4. Valved area of Cow jugular vein; Sagittal Plane Ultrasonography (A), Schematic presentation of ultrasonography (B). Valved area of Buffaloe jugular vein; Sagittal Plane Ultrasonography (A), Schematic presentation of ultrasonography (B). Jugular Vein (JV), Valve (arrows).

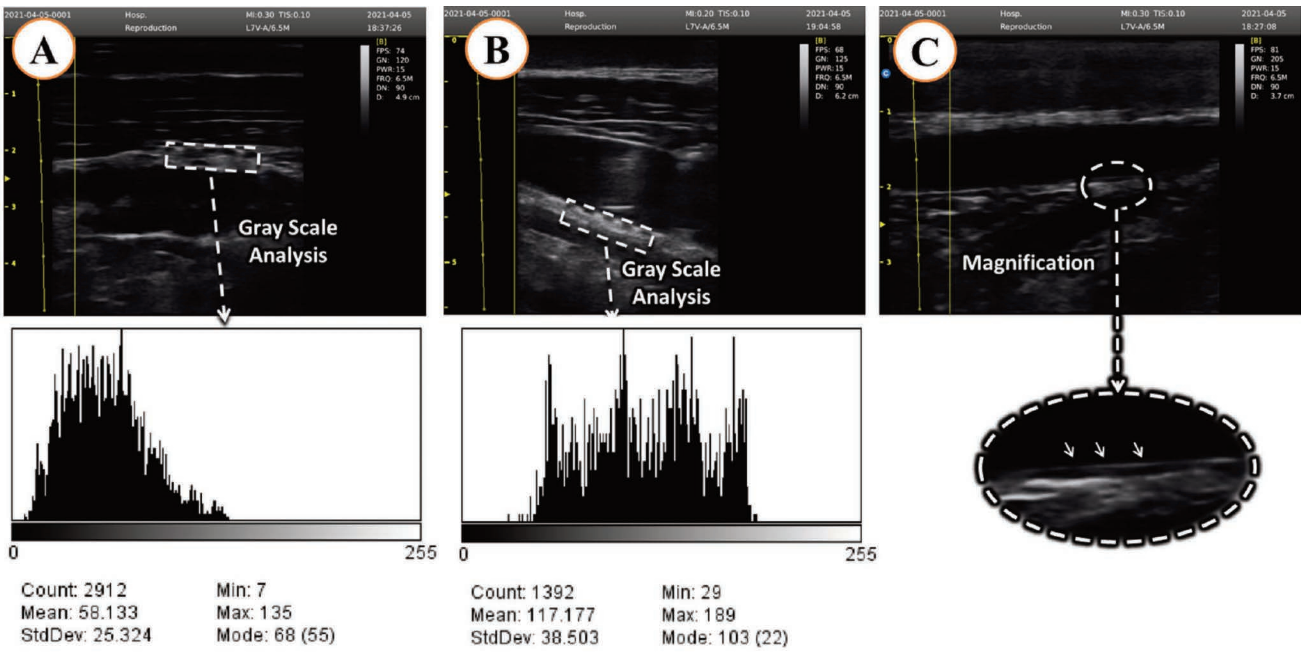

Fig. 5. Sagittal Plane Ultrasonography of cow jugular vein wall and its corresponding gray scale analysis (A), Sagittal Plane Ultrasonography of buffalo jugular vein wall and corresponding gray scale analysis (B), Magnification of bovine jugular vein ultrasonography revealed thin, hyper echoic wall and discrete borders of the endothelial surface (arrows).

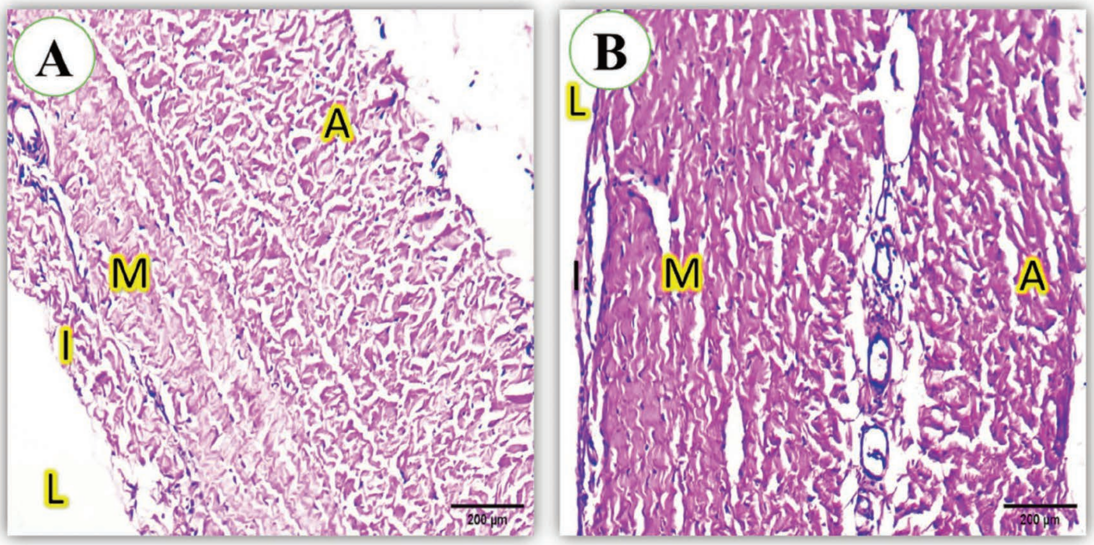

Fig. 6. Photomicrographs of L.S of cattle jugular vein (A) and Buffalo jugular vein (B). Showing the normal intima (I), media (M), and Adventitia (L: Lumen). Haematoxylin and Eosin.

jugular veins either collapsed or engorged was in agreement with Bain and Acvim (2013) and Bano *et al*. (2018). In current study, the jugular vein scanned by ultrasonography was round to oval (transverse plane) and tubular (sagittal plane) structure under the skin in the upper three quarters and get deeper in the 4th quarter. This result is in agreement with morphological finding in the current study and in the previous studies by Getty *et al*. (1975); Bain and Acvim (2013) and Bano *et al*. (2018). The former added that JV is covered by the skin and fascia and superficial to the carotid artery, from which it is separated in the anterior two thirds of the neck region by the omo-hyoideus muscle. Moreover, Sangwan and Mohindroo (2015) reported that prior to the thoracic inlet the JV get deeper between brachiocephalic muscles (dorsally) and sterno-cephalic muscle (ventrally). The entire anechogenicity of jugular vein lumen is attributed to filling with blood column, which considered the lowest density interface so appeared black anechoic in ultrasonography (Ihnatsenka and Boezaart, 2010).

The hyper echogenic venous wall is attributed to its fibro muscular components as discrete in histological examination performed in the present study. Sanchez-Hanke *et al*. (2000) and Ihnatsenka and Boezaart (2010) asserted that, tunica adventitia is the bulky thickest layer which composed of dense connective tissue fibers and appeared echogenic in ultrasonography due to its strong interfaces. Ultrasonographic and morphological examinations revealed that jugular vein owns its natural tricuspid valve; 2-3 in number and each one consists of three movable and very thin leaflets and the valves identified only at the last quarter. This finding is in acceptances with Dave *et al*. (2003) and Raja (2006) in bovine and Lu *et al*. (2008) in ovine. The later added that JV is also containing monocuspid and bicuspid valves. Moreover, in agreement with ultrasonographic examination in this study, Anwar *et al*. (2006) reported that the tricuspid appeared as three hypo echogenic leaflets attached to the endothelial lining of the vein. The hyoechogenicity of the tricuspid valve is resulted from its low dense structure from tunica intima only (Fath El-Bab, 2015). Regarding number and distribution of venous valves and in contrast to the obtained findings; Lu *et al*. (2008), stated that valves are distributed along the whole length of ovine jugular vein at (proximal, middle and distal segments) and added that the proximal venous segment, at all times, contained at least 2 valves and the other two segments may contain or not contain valves. Ultrasonographically, CVLW was significantly higher in the 2nd, 3rd and 4th quarter when compared with the first quarter. Morphological examination in the same hand revealed that; the shape of lumen is oval or round and its diameter is gradually increased along its length from cranial to caudal keeping the vein as conical shape structure. This result is in agreement with that described in human by Lorchirachoonkul *et al*. (2012) and Bano *et al*. (2018). Moreover, Magnano *et al*. (2016) added that the cross section area of JV was larger at the inferior cervical levels (toward the heart), than at superior cervical levels (toward the head). This result also is partially agreed with Lu *et al*. (2008), who divided the ovine neck into three segments (proximal, mid and distal) and the distal third lumen diameter was significantly higher than that of mid and proximal thirds. Ultrasonographic comparing between vein lumen width and vein wall thickness in cattle and buffaloes reveled; significantly wider vein lumen, thinner wall and lower wall gray scale in cattle than in buffalo.

Morphological examination revealed also; narrower vein lumen; thinner and less tough vein wall in cattle than in buffalo. Cattle wall lower gray scale analysis and lower toughness may be attributed to its lower wall density and thickness represented in thinner adventitia layer in cattle wall than buffalo wall (Boudjemline *et al*., 2003). The later added that wall of C-JV is soft and its softness makes it ideal for manipulation and suturing. Previous litratures have observed an individual and breed variation in respect to jugular vein lumen diameter and wall thickness within the same species of cattle (Schwarzwald and Jenni, 2009) and equine (Pasolini *et al*., 2019). The mean jugular vein lumen diameter of Brown Swiss cows were recorded to be 24.0±0.23 mm, which was significantly larger than those reported for Holstein cows (20.2±0.38 mm). Thus, ultrasonography assesment of live animal jugular vein features is considered an essential prerequisite for selection of suitable donor animal and suitable segment. Likewise; results of morphological examination performed in the current study concerning vein lumen diameter, vein wall thickness and venous valves situation and arrangement are closely similar to that assessed ultrasonographically. The clear similarities between both morphological and ultrasonographic examinations in the current research proved that ultrasonographic examination is the backbone of method for choosing an ideal jugular vein segment used as valved conduit with the maximum numbers of needed properties as ultrasonography act as a morphological mirror in live animal. Previous litratures ((Dave *et al*., 2003; Boudjemline *et al*., 2003; Li *et al*., 2007; Schwarzwald and Jenni, 2009, Koobatian *et al*., 2016; Dash *et al*. 2016) added that the perfect xenograft which could be used as a conduit segment must include its own natural valves, soft enough for easy handling and suturing, does not necessitate interposition of other materials for reconfiguration, possess a less thick wall, pliable and has excellent suturability and with lumen width within and up to 22 mm. Hence, the fourth quarter of cattle jugular vein possess most of these characters, it's the most appropriate segment used for post-mortem collection of jugular veins conduits at diameter lumen sizes within and up to 22 mm.

Conclusion

Cattle jugular vein has a less thick and less tough wall; and wider lumen when compared with buffalo jugular vein. The 4th quarter of cattle jugular vein is the most appropriate segment used for post-mortem collection of jugular veins conduits at diameter lumen sizes within and up to 22 mm. Ultrasonography seemed to provide an auspicious result as a prerequisite technique for choosing siutable animal and perfect segment which would be most appropriate for post-mortem collection of jugular veins conduits at diameter lumen sizes within and up to 22 mm.

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Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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