International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2024; 9(2): 780-783

www.veterinarypaper.com
Received: 02-01-2024
Accepted: 07-02-2024

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Comprehensive examination of buffalo diaphragm: Anatomical and histological insights

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DOI: https://doi.org/10.22271/veterinary.2024.v9.i2k.1292

Abstract
The study investigates the gross and histological characteristics of buffalo diaphragms to understand their role in digestive issues. Six diaphragm specimens from Ahmedabad Municipal Corporation Slaughterhouse were analyzed. Tissue samples were collected, preserved, and stained for histological examination. Results show a distinct attachment pattern of collagen fibers between tendinous and muscular parts of the diaphragm, notably looser compared to other musculotendinous junctions. Gross observation indicates a clear attachment at the abdominal surface, while the thoracic surface attachment is less prominent. These findings shed light on the unique anatomical features of buffalo diaphragms, which may contribute to digestive ailments. Understanding these structures is crucial for addressing health concerns in buffaloes and optimizing their role in the Indian economy.

Keywords: Buffalo, diaphragm, gross study, histology

1. Introduction
In India, buffaloes are fundamental to the dairy industry, serving as the backbone of milk production (Hegde, 2019) [1]. However, despite their critical role, buffaloes commonly experience digestive disorders, some of which may implicate the diaphragm. The diaphragm, serving as a vital musculotendinous barrier between the thoracic and abdominal cavities, plays a central role in respiration and facilitating essential physiological functions such as urination, defecation, and parturition (Mohindroo *et al*., 2007; and Athar *et al*., 2010) [2, 3].
Despite its significance, the buffalo diaphragm remains relatively underexplored in terms of its gross appearances, histology, thickness, and potential inflammatory changes. Understanding these aspects is essential for elucidating the mechanisms behind digestive disorders in buffaloes and improving husbandry practices to ensure the continued health and productivity of India's vital buffalo population.

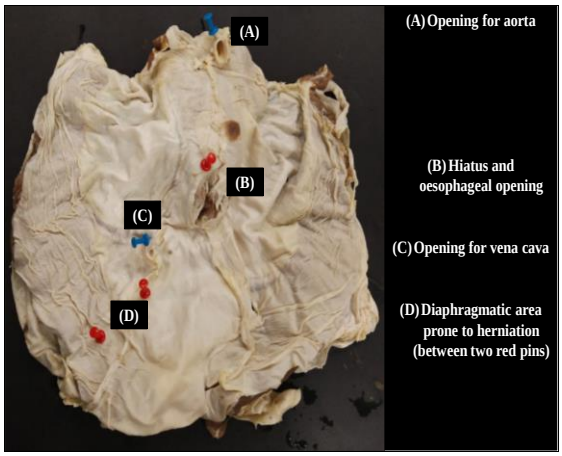
2. Materials and Methods
Six diaphragm samples from female buffaloes aged over one year were collected from the Ahmedabad Municipal Corporation Slaughterhouse, Jamalpur, Ahmedabad. The entire diaphragm was retrieved from the cadaver and preserved in chilled normal saline with gentamicin. At PGIVER, Kamdhenu University, Rajpur (Nava), Himatnagar, gross examination of the whole diaphragm specimen was conducted as depicted in Picture-01. Tissue samples measuring 1cm by 1cm were then collected from various areas of the diaphragm.

Picture 1: Diaphragm specimen (thoracic surface) obtained from a slaughtered buffaloes.

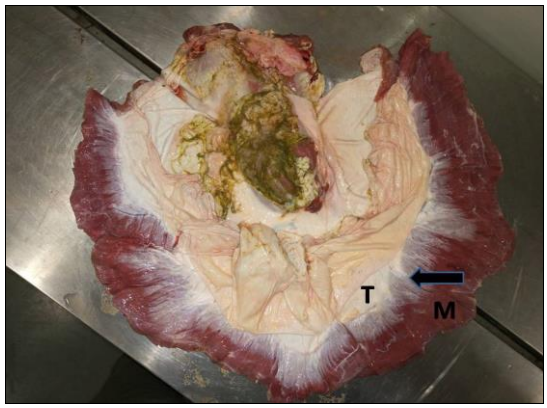
These tissue samples were promptly fixed and preserved in 10% neutral buffered formalin solution for at least 72 hours. Subsequently, they were transferred to the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, for further processing using routine paraffin techniques. Microscopic sections of 6-8 µm were prepared on glass slides and stained with Hematoxylin & Eosin following the method outlined by Singh and (Singh and Sulochana, 1997) [4].

3. Results & Discussion

Previous studies by (Athar *et al*., 2010; Talekar *et al*., 2018; Deshpande *et al*., 1981; and Sawale *et al*., 2015) [3, 5, 6, 7] have conducted exploratory laparorumenotomy and postmortem examinations to investigate diaphragmatic hernias in animals. Their findings have aided in identifying different openings and common sites of diaphragmatic defects, as depicted in Picture-02. Gross examination, after removing overlapping coverings, revealed a clear attachment of the tendinous part of the diaphragm to the muscular part on the abdominal surface, with less prominence on the thoracic surface, as illustrated in Pictures-03 & 04.



Picture 2: Location of different openings and area prone for herniation on diaphragm (thoracic surface)

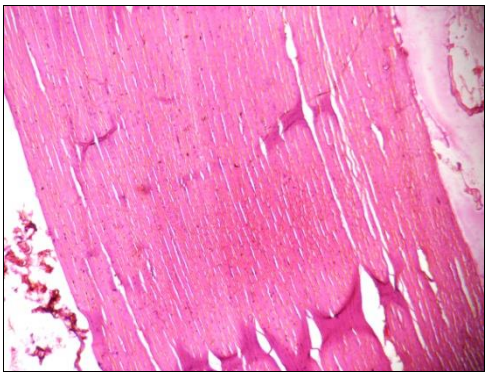


Picture 3: Abdominal surface of diaphragm after removal of its peritoneal covering muscular part (M) pure tendinous part (T) and musculotendinous attachment (arrow)

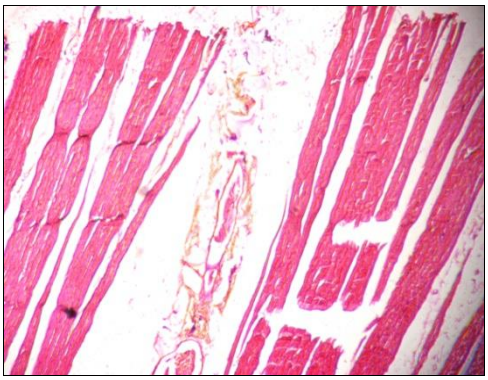
Picture 4: Thoracic surface of diaphragm after removal of plural covering showing muscular part (M) and tendinous part (T) but lack of musculotendinous attachment as shown in abdominal surface

While past pathological studies in cattle and buffaloes (Goedegebuure *et al*., 1983; Nakamura *et al*., 1994; Taib *et al*., 2016) [8, 9, 10] have revealed significant findings, our study did not uncover major pathological changes. Minor artifacts observed in histological images were attributed to technical processing errors. This study contributes to the broader understanding of diaphragmatic anatomy and pathology in animals, highlighting the need for meticulous technical procedures in histological analysis.

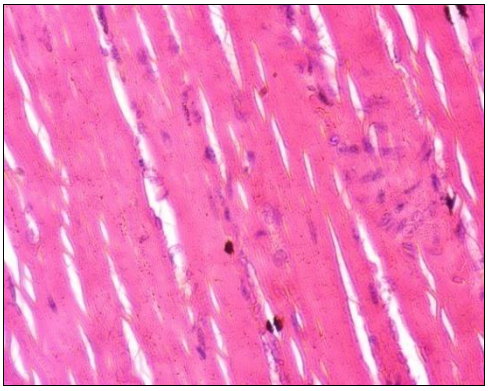
The muscular part of the diaphragm exhibited closely arranged, long, cylindrical skeletal muscle fibers with multiple peripheral nuclei and striations, consistent with findings by (Sathapathy *et al*., 2015) [11]. This histological appearance is depicted in Picture-05. Additionally, Picture-06 illustrates splitting of muscle fibers with transfer rupture of muscle tissues on either side of blood vessels, while Picture-07 highlights the cross-striations of skeletal muscles. These observations provide valuable insights into the structural characteristics of the diaphragmatic musculature, enhancing our understanding of its functional properties and potential implications for physiological processes such as respiration.



Picture 5: Section of the muscular part of diaphragm with parallel arrangement of skeletal muscles fiber which stained pink with eosin with peripherally blue colored nuclei (4X View)

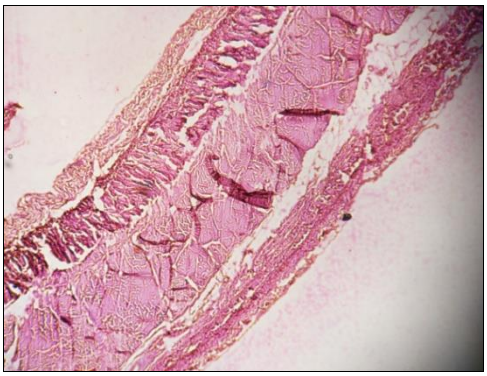


Picture-06: Section of muscular part of diaphragm showing splitting of muscle fibers with transfer rupture of the muscle tissue on either side of blood vessels (4X View)

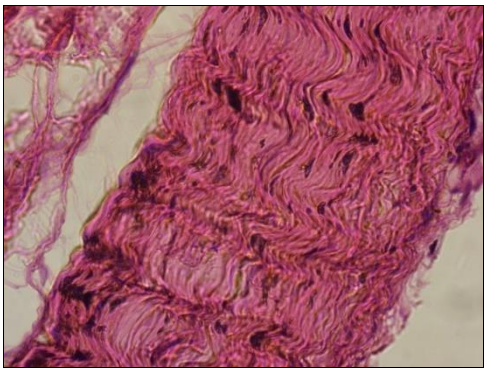


Picture 7: Skeletal muscles of diaphragm showing cross striations (40X View)

The tendinous part of the diaphragm exhibited wavy bundles of collagen fibers intermingled with muscle fiber bundles oriented in various directions, alongside the presence of blood vessels and nerve supply, consistent with findings by (Ravali *et al*., 2018) [12]. Picture-08 illustrates a cross-section of collagen bundles within the central areas covered on either side by serous membrane. In Picture-09, a densely packed arrangement of collagen bundles in the tendinous part of the diaphragm is clearly visible.



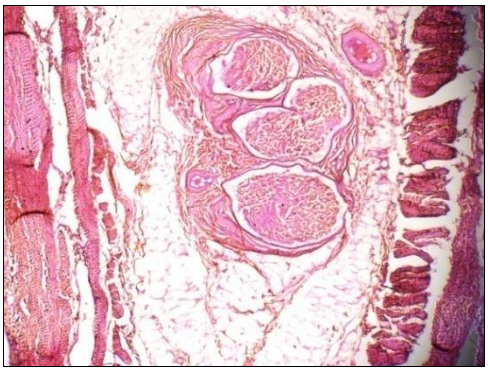
Picture 8: Section of tendinous part of diaphragm showing cross section of collagen bundles into the central areas covered either side by serous membrane. Left side - pleura (thoracic face) and right side - peritoneum (abdominal face) (4X View)



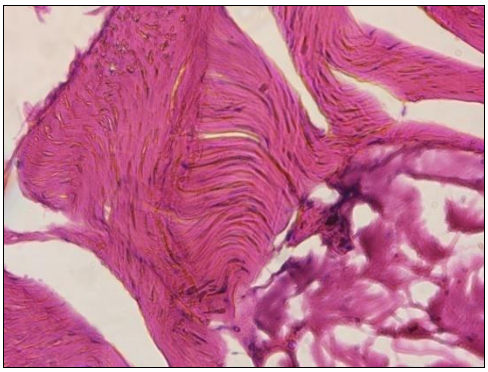
Picture 9: Section of tendinous part of diaphragm showing densely packed arrangement of collagen bundles (40X View)

Picture-10 depicts a branch of the phrenic artery supplying the diaphragm, while Picture-11 shows a section of the phrenic nerve containing neurofibrils and surrounding structures. Furthermore, Picture-12 highlights the presence of skeletal muscles and bundles of collagen fibers at the musculotendinous part/junction of the diaphragm. These observations contribute to our understanding of the structural composition and functional properties of the diaphragm, shedding light on its role in physiological processes such as Respiration and abdominal support.

Picture 10: Section showing branch of phrenic artery supplying the diaphragm showing all the three distinct tunica intima(A), tunica media(B) and tunica externa(C) layers (20X View)



Picture 11: Section of the phrenic nerve showing the neurofibrils in bundles and surrounded by perineurium in a large lose connective tissue area (4X View)



Picture 12: Section of musculotendinous part/junction showing skeletal muscles and bundles of collagen fibers (20X View)

4. Conclusion

The comprehensive examination of the diaphragm in buffaloes conducted in this study contributes significantly to the broader understanding of diaphragmatic anatomy and pathology in animals. Gross examination emphasized the clear attachment of the tendinous part of the diaphragm to the muscular part on the abdominal surface. While past pathological studies in cattle and buffaloes have revealed significant findings, our study did not uncover major pathological changes, attributing observed artifacts to technical processing errors.

The histological examination of the muscular part of the diaphragm revealed closely arranged skeletal muscle fibers with characteristic peripheral nuclei and striations, providing valuable insights into its structural composition and functional properties. Similarly, the tendinous part exhibited wavy collagen bundles intermingled with muscle fibers shedding light on its structural complexity. Furthermore, the presence of blood vessels, nerve supply, and the depiction of the phrenic artery and nerve further contribute to our understanding of the diaphragm's role in physiological processes such as respiration and abdominal support.

Overall, this study contributes to the broader understanding of diaphragmatic anatomy and pathology in animals, emphasizing the necessity of meticulous technical procedures in histological analysis. These findings not only enhance our comprehension of the diaphragm's role in physiological processes but also offer valuable insights into potential avenues for further research and clinical applications in animal health and husbandry practices.

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