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Comprehensive study on ultrasonographic evaluation of buffalo diaphragm

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Abstract
Buffalo farming is crucial for India's economy, but buffaloes commonly face digestive issues, potentially involving the diaphragm. This study aimed to evaluate diaphragm thickness using ultrasonography (USG) and assess age-related changes. Diaphragm samples were obtained from slaughtered buffaloes, clinically healthy ones, and those with clinical signs of diaphragmatic issues. Measurements were taken using vernier caliper and organ bath USG. Three groups were formed: group-I (cadaveric diaphragms, n=6, age >1 year), group-II (clinically healthy, n=6, age 1 to 2 years), and group-III (clinical cases, n=15, age <4 years). Tendinous and musculotendinous junctions couldn't be assessed clinically. Mean ultrasonographic thickness of the muscular diaphragm was 12.58±1.16 mm (group-I), 5.22±0.50 mm (group-II), and 9.73±0.53 mm (group-III). Variations may stem from diaphragm detachment, animal age, housing and feeding practices, diseases, stomach content, and movement during examination.

Keywords: Buffalo, diaphragm, ultrasonography, thickness

1. Introduction
Buffalo husbandry is a cornerstone of India's agricultural economy, with buffaloes often dubbed as the nation's primary "milking machine" (Hegde, 2019) [5]. However, despite their pivotal role in bolstering the economy, buffaloes frequently encounter digestive ailments, some of which may implicate the diaphragm (Mohindroo *et al*., 2007; Athar *et al*., 2010; Priyanka and Bisla, 2019) [11, 1, 12].

The diaphragm, a musculotendinous structure positioned between the thoracic and abdominal cavities, serves as a vital anatomical barrier, separating organs and structures of the thorax from those of the abdomen (Mohindroo *et al*., 2007; Athar *et al*., 2010) [11, 1]. Its primary function lies in respiration, facilitating inhalation through contraction and exhalation through relaxation. Additionally, diaphragmatic contractions contribute to increased intra-abdominal pressure, aiding processes like urination, defecation, and parturition (Ghosh, 2018) [4].

Despite its critical physiological functions, the buffalo diaphragm remains relatively understudied, particularly concerning its thickness, ultrasonographic features, and radiological characteristics. Diagnosing diaphragmatic affections poses challenges due to the diaphragm's anatomical position. While radiography and ultrasonography (USG) have been utilized for diagnosing digestive disorders in cattle and buffaloes, USG presents as a promising modality for visualizing soft tissue structures like the diaphragm (Misk and Semeika, 2001; Athar *et al*., 2010; Braun *et al*., 2018; Lakhpati *et al*., 2019) [10, 1, 3, 9]. This study aims to fill this gap by exploring various aspects of the buffalo diaphragm, thereby enhancing diagnostic capabilities and improving the management of diaphragmatic disorders in buffaloes.

2. Material and Methods
2.1 Selection of animals and slaughterhouse specimen
Selection of the animals was carried out in three major groups in which Group-I include 6 number slaughter buffalo cadaver which are having the age more than 1 year, Group-II includes 6 numbers of clinical healthy animals which are having the age from 1 to 2 years, group-III includes the 15 clinical cases of peri-diaphragmatic affections.

2.1.1 Group-I (Slaughtered animals; n=6; Age >1 year)
Six diaphragm specimens from cadaver of clinically healthy and slaughtered buffaloes were collected from Slaughterhouse, Ahmedabad Municipal Corporation, Jamalpur [Picture-01].

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

2.1.2 Group-II (Clinically healthy animals; n=6; Age 1 to 2 years)

Six clinically healthy buffaloes of Livestock Instructional Farm, Kamdhenu University, Rajpur (Nava), Himmatnagar-383010 were included in Group-II.

2.1.3 Group-III (Clinical cases of peri-diaphragmatic affections; n=15; Age <4 years)

Cases presenting with symptoms suggestive of diaphragmatic or peri-diaphragmatic affections were admitted to the Veterinary Hospital at PGIVER. Following the acquisition of owner consent, these cases underwent thorough investigations to confirm the presence of diaphragmatic issues. The diagnostic process included anamnesis, clinical examination, ferroscopy, and ultrasonography (USG), adhering to established protocols. Subsequently, a total of 15 buffaloes diagnosed with diaphragmatic or peri-diaphragmatic affections were identified and categorized under Group-III for further diagnostic imaging procedures.

2.2 Examination of Diaphragm specimen from Slaughterhouse

2.2.1 Measurement of diaphragmatic thickness

A diaphragm specimen from cadaver of normal Buffaloes were collected from slaughtered animals and measurement of its thickness was carried out using a vernier caliper [Picture-02].

Picture 2: Measurement of diaphragmatic thickness using vernier calipers

2.2.2 Organ bath (*in vitro*) USG examination

An organ bath study was conducted on the slaughtered diaphragm specimens to investigate their echo texture. The findings from this *in vitro* standardization were then compared with those obtained from *in vivo* ultrasonography (USG) [Picture-03 and 04]. The *in vitro* standardization of the diaphragm was performed following the method outlined by Imran, 2010 [6]. Diaphragm samples, either whole or in pieces for convenience, were collected from the cadaver and stored in chilled (4 °C) normal saline solution containing 0.048% gentamicin. These samples were then placed in a water bath and subjected to repeated ultrasonography using a 3.5 MHz curvilinear transducer.

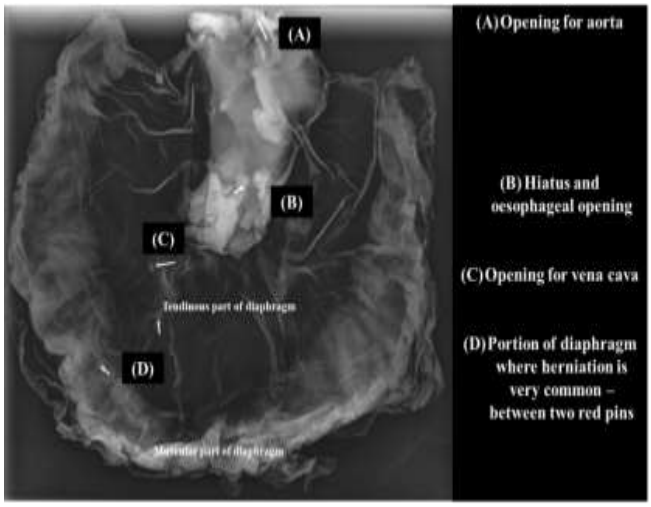
While organ bath USG has been previously utilized for learning the USG features of various abdominal organs in bovines (Imran, 2010; Imran *et al*., 2011; Barreiro-Vázquez *et al*., 2020) [6-7, 2], there is limited literature available regarding the application of organ bath USG specifically for the diaphragm in buffaloes.

Picture 3: Preparation of slaughtered diaphragm specimen for Organ bath (*in vitro)* USG examination

Picture 4: Organ bath *in vitro* USG being performed on diaphragm

2.2.3 Radiography

Slaughterhouse specimens of diaphragm were subjected to radiographic examination [Picture-05] to examine normal radiographic appearance of buffalo's diaphragm and its parts.



Picture 5: Radiographic appearance of diaphragm (thoracic surface)

2.3 *In-vivo* ultrasonography of diaphragm

Ultrasonographic examinations were conducted on non-sedated animals from Groups II and III, utilizing the Esaote Mylab 40 Vet Ultrasound System. The examinations were performed with a 3.5 MHz convex transducer while the animals stood in a natural position. The diaphragm was examined by positioning the transducer parallel to the ribs on both sides of the sternum, as well as towards the left and right lateral thorax, covering the area from the 9th to 5th intercostal spaces up to the level of the elbow. Sequential examinations were carried out from the left side, midline, and then from the right side, respectively.

B-mode display settings were employed for the ultrasonographic evaluation of abdominal organs, following the recommendations outlined by Kaske *et al*., 1994 [8]. This standardized approach ensured consistent and accurate assessment of the diaphragm and other abdominal structures in the non-sedated animals.

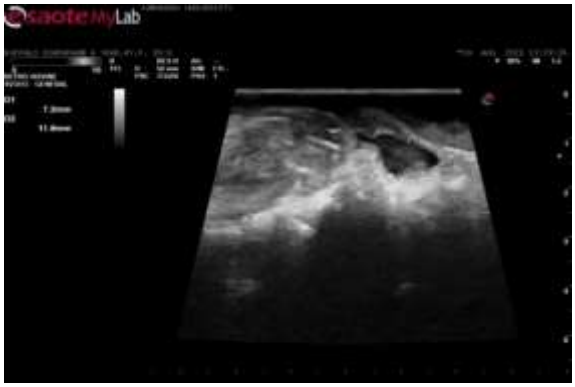
3. Results and Discussion

The diaphragm of buffaloes is composed of tendinous, muscular, and musculotendinous junction components, with three openings for the pulmonary artery, esophagus, and posterior vena cava, as previously described by Athar *et al*., 2010 [1]; Singh, 2016 [13]; and Ghosh, 2018 [4]. The ultrasonographic images provided in Pictures-06 to 11 offers visual insights into the different parts of the diaphragm, aiding in its characterization and identification during diagnostic procedures.

Picture 06: Organ bath USG image of the muscular part of diaphragm.

Picture-07: Organ bath USG image of the tendinous part of diaphragm

Picture-08: Organ bath USG image of the musculotendinous part/junction of diaphragm



Picture-09: Organ bath USG of diaphragm showing opening for aorta

Picture-10: Organ bath USG of diaphragm showing opening for vena cava

Picture-11: Organ bath USG of diaphragm showing opening for esophageal hiatus

The comparison of diaphragm thickness across different measurement techniques, as presented in Table-01 and 02, highlights variations in measurement values obtained via vernier calipers, organ bath ultrasonography (*in vitro*), and *in vivo* ultrasonography. These differences may arise due to factors such as specimen preparation, imaging methodology, and anatomical variations among individual animals.

Table 1: Thickness of different parts of diaphragm samples obtained from slaughterhouse (Mean±S.E. values in mm).

Group No.	**I**	
Samples/Animals	**Diaphragm from slaughterhouse**	
Criteria	**Buffalo (>1 year)**	
No.	**06**	
Particulars of diaphragmatic thickness	**Vernier calipers (mm)**	**Organ bath USG (mm)**
Muscular part	12.37±1.13 mm	12.58±1.16 mm
Tendinous part	2.5±0.11 mm	2.6±0.13 mm
Musculotendinous part/junction	6.55±0.72 mm	6.78±0.92 mm

While organ bath ultrasonography has been previously utilized for learning the ultrasonographic features of abdominal organs in cattle (Imran, 2010; Imran *et al*., 2011; Barreiro-Vázquez *et al*., 2020) [6-7, 2], there is limited literature available regarding its application for studying the diaphragm in buffaloes. Further research in this area could provide valuable insights into the ultrasonographic characteristics of the buffalo diaphragm, enhancing diagnostic capabilities for diaphragmatic disorders in these animals.

Table 2: Thickness (Mean±S.E. values in mm) of diaphragm in animals under Groups-I, II & III

Group No.	I	II	III
Samples/Animals	Slaughtered buffaloes	Clinically healthy animals	Clinical cases
Criteria	Buffalo (>1 year)	Buffalo (>1 to <2 years)	Buffalo (> 4 years)
No.	06	06	15
Particulars of diaphragmatic thickness	Organ bath USG (mm)	USG (mm) (at 5th to 8th ICS)	USG (mm) (at 5th to 8th ICS)
Muscular part	12.58±1.16 mm	5.22±0.50 mm	9.73±0.53 mm
Tendinous part	2.6±0.13 mm	Not accessible	Not accessible
Musculotendinous part/junction	6.78±0.92 mm	Not accessible	Not accessible

While organ bath ultrasonography has been previously employed for the initial learning of ultrasonographic features of abdominal organs in cattles and buffaloes (Imran, 2010; Imran *et al*., 2011; Barreiro-Vázquez *et al*., 2020) [6-7, 2], its application for the diaphragm in buffaloes remains underexplored. The limited literature on organ bath USG of the diaphragm underscores the novelty and significance of our study in advancing the understanding and diagnostic capabilities related to diaphragmatic affections in buffaloes.
Additionally, the diverse ultrasonographic observations obtained during *in vivo* examinations, as illustrated in Pictures-11 to 21, underscore the diagnostic utility of ultrasonography in evaluating the diaphragm and other abdominal structures in buffaloes.

Conclusion

Our study provides a comprehensive evaluation of the ultrasonographic features and measurements of the buffalo diaphragm, reaffirming its anatomical structure and diagnostic potential. Through meticulous examination, we confirmed the established structure of the buffalo diaphragm and accurately measured its muscular part thickness using *in vivo* ultrasonography. Our findings underscore the reliability of ultrasonography as a diagnostic tool for evaluating the diaphragm in buffaloes and highlight the potential of organ bath ultrasonography, an area previously underexplored. This research advances our understanding of buffalo diaphragm anatomy and ultrasonographic characteristics, laying the groundwork for improved diagnostic approaches in veterinary practice.

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