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Research Article

Evaluation of Clinical, Hemato-Biochemical and Ultrasonographic Findings in Egyptian Buffaloes with Diaphragmatic Hernia

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ABSTRACT

The present study was designed to evaluate diaphragmatic hernia (DH) in buffaloes with special emphasis on the hemato-biochemical alterations and diagnostic ultrasonography compared with the clinically healthy ones. Twelve buffaloes with a history of anorexia, tympany, reduction of milk production with no response to previous medical treatment were included in the present study in addition to 10 clinically healthy buffaloes were used as a control group. These animals were subjected to thorough clinical examination and the positive cases were further evaluated by hemato-biochemical analysis, which revealed significant elevation in total leucocytes, total proteins, globulin and Haptoglobin (Hp), Serum amyloid A (SAA) and Fibrinogen (Fb) in comparison with the control group. Ultrasonographically, reticulum of all buffaloes with DH was detected at the level of 4^{th} /5^{th} intercostal space (ICS).
As there is no substitution for clinical examination, but it is not specific in this condition as it is similar in many other diseases, so the using of ultrasonography is essential for proper evaluation. Hemato-biochemical analysis is of additional value in the diagnosis of such condition.

Key words: Diaphragmatic hernia, Ultrasound, Acute phase proteins, Buffaloes

INTRODUCTION

Diaphragmatic hernia (DH) is a serious thoraco-digestive disorder in which a part abdominal viscera "mainly reticulum" passed into the thoracic cavity through a congenital or acquired opening in the diaphragm causing chronic ruminal tympany, anorexia and displacement of the heart (Radostits *et al*., 2007). It is a chronic wasting and inflammatory disorder in adult buffaloes (Bisla *et al*., 2002).

Buffaloes are susceptible to DH than other ruminant species and this due to the anatomical differences of buffaloes diaphragm. A relatively small tendinous portion of the diaphragm resulting in innate weakness, making this species more prone to such condition (Singh *et al*., 2006). The incidence of DH is higher in buffaloes than in cattle. There are multifactorial causes behind these differences. The size of the abdomen, animal activity as buffaloes are more active than cattle. Buffaloes usually have a tendency for swimming, jumping which add an additional pressure over the diaphragm. Absence of pericardiophrenic artery supplying the lower part of the diaphragm in buffaloes all these factors contribute to the incidence of the disease. Lesser collagen content in diaphragm of buffaloes than cattle making it less elastic (Singh *et al*., 2006). Sharp foreign bodies are incriminated as the main cause of DH in cattle and buffalo. As it penetrates the diaphragm induce diaphragmatitis, weakness and diaphragmatic rupture which in turn, leads to herniation of abdominal viscera (mainly a segment of the reticulum, sometimes abomasum) into the thoracic cavity resulting in DH (Divers and Peek, 2008 and Athar *et al*., 2010).

Diaphragmatic hernia is a devastating problem as it causes a high economic losses especially when there is no either medical or surgical treatment for this condition. Few Indian authors mentioned a treated case of DH, however, no other authors confirmed these results (Saini *et al*., 2007). Diaphragmatic hernia has a wide range of clinical signs depending on the size and the type of viscera herniated (Kelmer *et al*., 2008). The animals showed general signs of indigestion including partial or complete anorexia, ruminal recurrent tympany, scanty faeces, reduced milk production and atonized rumen with suspended rumination which are considered general signs in buffaloes with DH and other many digestive disturbances (Saini *et al*., 2007; Abdelaal *et al*., 2009 and Misk, 2015). So the diagnosis of DH is erratic and

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difficult. Diaphragmatic hernia should be suspected if the animal presents with dyspnoea, bilateral asymmetric lung sounds with sounds of forestomach (Singh *et al*., 2006 and Kelmer *et al*., 2008).

Acute phase proteins (APPs) are sensitive factors that allow the early detection of inflammation in ruminants (Kirbas *et al*., 2015). The most important AAPs proteins in cattle are haptoglobin (Hp), serum amyloid A (SAA), fibrinogen (Fb) (Eckersall & Conner, 1988 and Horadagoda *et al*., 1993).

Haptoglobin, SAA and Fb are considered the most important and useful indicators of inflammatory processes (Gonzalez *et al*., 2008 and Eckersall and Bell 2010), their serum levels could reflect the degree of tissue damage in the diseased animal (Murata *et al*., 2004).

According to the author, few reports are available for diagnosis of DH in buffaloes. Buffaloes with DH may be presented with a history pointing to other disease. So case history and clinical examination are not sufficient for definite diagnosis of DH (Narale and Bhokre, 2004). So advanced diagnostic techniques are required especially in buffalo as it is mentioned to express fewer signs of pain than cattle (Saleh *et al*., 2008). Ultrasonography is a non-invasive imaging technique and it has been emerged as a reliable tool and method of choice for diagnosis of DH (Flöck, 2004 and Mohamed, 2010). The visibility of reticular wall and its motility at the level of the 5th ICS is considered positive DH. However, In late pregnant buffaloes a greater pressure is applied by the gravid uterus over the diaphragm and unherniated reticulum can be seen at the level of the 5th ICS making a false ultrasonographic positive result (Kumar and Saini, 2011). Therefore, the aim of the present study is to throw a light on the clinical, hematobiochemical and uses of ultrasonography as field technique for diagnosis of DH in buffaloes with special reference to AAPs alterations in such condition.

MATERIALS AND METHODS

Animals

Twelve buffaloes were referred to Zagazig Veterinary Teaching Hospital between March, 2013 and May, 2015 due to anorexia. The animals were 4-9 years of age and their weight ranged between 350 to 500 kg. Three buffaloes were pregnant over 7 months, six were recently calved (less than 5 weeks) and three were non pregnant. Animals were with a history of anorexia, digestive disturbances in form tympany, scanty feces with reduction of milk production. Animals were with a past history of receiving various medical treatments with no response. Additionally, 10 healthy animals were included in this study as a control group.

Clinical assessment: All animals were subjected to thorough clinical examination with application of various pain tests according to the method described by Dirksen *et al*. (1990). Data concerning sex, pregnancy, parturition, appetite, milk yield, general attitude, pain expression (grunting, tearing), eye appearance (conjunctival mucous membranes and sclera blood vessels), body temperature, respiration rates, heart sound and ruminal movement were recorded.

Blood Sampling and Biochemical Assay

Whole blood and serum samples were collected from each animal by jugular vein puncture, 3 ml of blood was transferred into vacuum EDTA coated tubes "for hematological examination, Hb, PCV, TEC, TLC which were estimated within 2 - 4 hours of collection using blood cell counter machine and plasma fibrinogen. The remaining blood was kept in slope position for serum separation according to Kanekeo *et al*. (1997). The Serum glucose, total proteins, albumin, globulin and liver enzymes (AST and ALT) were estimated by standard procedures using (Diagnostic Zrt. Commercial kits) which were provided by Egyptian Company for Biotechnology, Cairo, Egypt, and the reading was taken by spectrophotometer. The concentration of Hp was assessed photometrically using a quantitative hemoglobin binding assay modified after Elson (1974). Serum amyloid A was analyzed by the method of immunosorbent assay (ELISA) using commercial ELISA kit.

Ultrasonographical examination

All the animals were subjected to lateral right- left ultrasonographic examination of reticulo-thoracic region and the results were recorded. The reticulum and surrounding structures "especially lung and heart" were examined using 3.5 MHz convex transducer as described by Braun and Gotz (1994). The examined area from 3rd to 8th intercostal space from left, right and ventral midline area was prepared by clipping of hair and application of coupling gel. The scanning of the reticulum in cases of DH in adult buffaloes was done at 5th intercostal space (Neeraj *et al*., 2013). The lung area was examined with the transducer probe held parallel to the 3rd to 11th intercostal space. Reticular wall was evaluated ultrasonographically "its shape, thickness, frequency of biphasic contraction/2 minutes" with comparing of reticular motility in the abdominal and thoracic cavity in buffaloes suffered from DH.

Statistical analysis

Data were analyzed using packaged SPSS program for windows version 10.0.1 (SPSS Inc., Chicago, IL). All data were presented mean ± standard error (SE). Differences between groups were determined by LSD Post hoc test. The significance level was set at $P<0.05$.

RESULTS AND DISCUSSION

Incidence and clinical findings

Diaphragmatic hernia is considered as a serious thoraco-abdominal disorder among buffaloes compared with other ruminant species. In the present study 12 buffaloes had a DH. Higher prevalence of DH in buffaloes versus relatively lower prevalence in cows and this may be attributed to the lesser collagen content, elasticity, and vascularity of the buffalo diaphragm (Singh *et al*., 2006). Age of affected animals was ranged from 4-9 years old. Three were pregnant (25%), 6 were recently calved (50%), 3 were non-pregnant (25%). The duration of illness ranged from five days to four weeks. Pain test was positive in 9 cases (75%) which indicate that foreign body syndrome is the main cause of DH in buffaloes due to their swallowing habit, although other factors as increased

intra-abdominal pressure due to ruminal tympany, heavy pregnancy, violet fall, chronic cough and straining in parturition has a contributing role in the occurrence of the disease (Singh *et al*., 2006 and Abouelnasr *et al*., 2012).

Clinical findings of the examined animals were shown in (Table 1); all buffaloes were anorexic, depressed, with a history of weight loss and decrease in milk production. Buffaloes were dehydrated with ruminal atony "ruminal motility was reduced in 7 cases and in the other 3 cases there was no ruminal motility" which is considered a sign of indigestion and this may be attributed to the restricted contraction of the herniated reticulum. These findings were in accordance with those reported previously (Singh *et al*., 2006; Athar *et al*., 2010 and Abdelaal *et al*., 2014). Although 2 cases revealed hyper motility and this may be due to the involvement of the vagus nerve, which passes through the diaphragm (Andrews *et al*., 2004). Although Saini *et al*. (2007) reported that ruminal movement was within the normal range in cows with DH. Animals had been treated unsuccessfully with antibiotics, antibloat agents and stomachics.

The animals showed congested mucous membrane and engorgement of scleral blood vessels. In some cases there was regurgitation of ingesta "3 cases" and this may be due to impaired process of eructation and rumination due to herniation of the reticulum as it has an important role in eructation and rumination process. Tachypenia and dyspnoea appeared in all diseased cases as in (Fig. 1). Lung sounds were normal in some cases and reduced in other cases and sometimes absent and systolic murmurs may be present, similar results obtained by Athar *et al*. (2010) and Bellavance *et al*. (2010).

Rectal temperature was within the normal range while bradycardia were recorded, similar results were previously recorded by Athar *et al*. (2010); Bellavance *et al*. (2010) and Aref and Abdel-Hakiem (2013), bradycardia in this study may be attributed to displacement of the heart away from the chest wall due to reticular herniation.

The duration of illness, size and location of the diaphragmatic tear and the amount of compromised viscera play a significant role in the prognosis of DH (Saini *et al*., 2000). Regarding the hemato-biochemical findings of diseased buffaloes, there was a leukocytosis, significant increase in total proteins, globulin, APPs (HP, SAA and Fb) and hepatic enzymes (AST&ALT) when compared with controls (Table 2) similar findings recorded previously by Bellavance *et al*. (2010); Aref and Abdel-Hakiem (2013) and Abdelaal *et al*. (2014). Generally, this result was recorded in cattle with chronic inflammatory conditions, although Saini *et al*. (2007) recorded that leucocytes were within the reference limit. While there was a significant decrease of TEC and Hb. content, similar results obtained by Aref and Abdel-Hakiem (2013) and this indicates anemia which may be attributed to damage to the diaphragm or the chronic inflammatory process (Ocal *et al*., 2008). Increased PCV % may be attributed to dehydration due to the long period of anorexia. A significant decrease of glucose may be attributed to anorexia. These changes in serum proteins 'hyperproteinemi, hypoalbuminemia and hyperglobinemia' are a reflection of cellular to tissue destruction and inflammation (Gruys *et al*., 1994).

Acute phase proteins measured in this study were significantly elevated compared with the control buffaloes. Levels of acute phase proteins, especially HP and SAA may be better able to differentiate between acute and chronic inflammation than hematological tests (Horadagoda *et al*., 1999 and Nazifi *et al*., 2008). As SAA increased mainly in chronic inflammation while Hp elevated in acute inflammation and decreased by recovery or chronicity (Gruys *et al*. 2005 and Petersen *et al*. 2004). However, the level remains high in chronic cases, if stimulation continues (Bozukluhan and Gokce, 2007 and Petersen *et al*. 2004).

Table 1: Clinical findings of buffaloes with DH in comparison with clinically healthy buffaloes**:**

Clinical findings	Control group	DH group		
		Abnormal findings	No. of animal	Percent %
Appetite	Good	Reduced- anorexia	12	100
Recurrent tympany	Negative	Positive	5	41.66
Persistent tympany	Negative	Positive	7	58.33
Feces	Semi-solid	Scanty hard	8	66.66
		Scanty soft	2	16.66
		Diarrhoea	2	16.66
Regurgitation of food from mouth	Negative	Positive	3	25
		Negative	9	75
Dyspnoea	Negative	Dyspneic	12	100
Pain test	Negative	Positive	9	75
		Negative	3	25
Ruminal movement/ 2minutes	3.00±0.3	Decreased "0 .7±0.2"	7	58.33
		Stasis	3	25
		Increased "6-7/2min"	2	16.66
Abnormal lung sound	Negative	Positive	4	33.33
		Negative	8	66.66
Systolic heart sound	Negative	Positive	3	25
		Negative	9	75
Respiration rate/minute.	24.8±.37	36.4±1.0	P Value	
			0.000	
Rectal temperature	38.48±0.03	38.34±0.13	0.351	
Heart rate/minute	66.6±1.12	43.20±4.22	0.001	

* P is significant at < 0.05

Fig. 1: Dyspnoea in a buffalo with DH, notice opening of the mouth and protrusion of tongue.

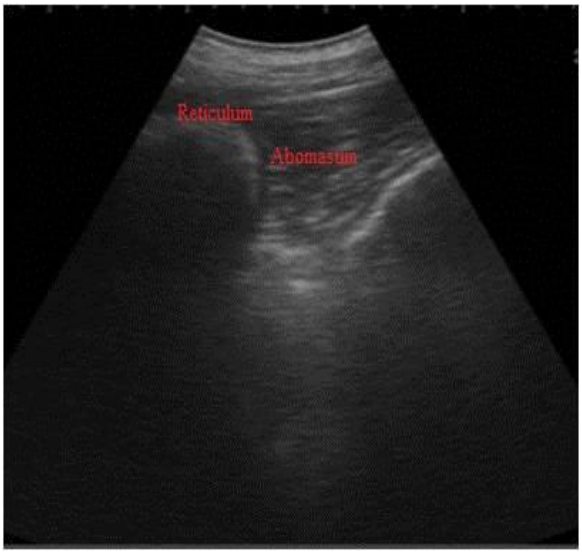

Fig. 2: Sonogram of normal buffalo's reticulum, imaged from ventral midline of the abdomen. Notice the half-moon shape of the reticular wall.

Table 2: Hemato-biochemical data of buffaloes with DH in comparison with clinically healthy buffaloes**:**

Parameter	Control group (n=10)	DH group (n=12)	P Value
TEC 10^6/ml	6.8±0.18	5.1±0.1	0.000
TLC10^3/ml	6.6±0.4	13.16±1.2	0.001
Hb. g/dl	11.26±0.24	8.18±0.13	0.000
PCV%	30.1±0.65	35.8±1.53	0.009
Glucose mg/dl	60.6±1.4	42.4±1.4	0.000
Total proteins g/l	6.4±0.2	7.5±.12	0.010
Albumin g/l	3.8±0.2	2.74±0.08	0.003
Globulin g/l	2.6±0.15	4.8±.094	0.000
Hp g/l	0.03±0.01	1.14±0.1	0.000
SAA ug/ml	65.0±1.7	163.20±2.817	0.000
Fb mg/dl	246.8±21.8	605.8±44.2	0.000
AST iu/l	74.2±1.68	159.0±6.55	0.000
ALT iu/l	32.80±1.43	65.0±2.92	0.000

* P is significant at <0.05

Fibrinogen is commonly measured in ruminants as it is the best indicator of inflammation because fibrinogen concentrations often increase prior to leucocytes alterations (Latimer *et al*., 2003 and Jones and Allison, 2007). Fibrinogen level was significantly increased and this may due to increase fibrin content as herniated part of the reticulum was adhered all around the ring of the diaphragm (Athar *et al*., 2010). The significant elevation of serum AST and ALT indicate the involvement of the liver in the pathogenesis of the disease and this may be attributed to fatty liver, which occurred consequently to anorexia, as all animals under investigations were anorexic for long period, causing fatty liver with leakage of these enzymes in the blood, similar results obtained by Öcal *et al*. (2008) and Bellavance *et al*. (2010). Although AST and ALT enzymes are specific for liver affections, the change of AST level also reflects muscle affection "diaphragm" as it is muscle specific enzyme rather than being a liver specific enzyme.

In conclusion, the changes in hematological values "leucocytosis" and biochemical parameters (low concentrations of albumins together with high Hp, SAA, Fb and AST) are suggestive of inflammatory changes in the body.

Ultrasonographical findings

Ultrasonographic evaluation reveals a portion of forestomachs "reticulum" in the thoracic cavity that has herniated through the rent on ventrolateral diaphragm at the musculotendinous junction. Reticulum was normally scanned as a crescent shaped "half-moon" structure with smooth contour (Fig. 2) and biphasic contraction. The peak of the first contraction curve could be visualized while the peak of the second contraction was out of the depth capacity of 15cm screen.

Diaphragmatic hernia was diagnosed by placing the transducer at 3rd - 5th ICS of left and right sides of the thorax. Appearance of reticular wall "the half-moon shape, relatively straight line in some cases (Fig. 3b and 3d) at this position strongly suggesting DH and this was in agreement with the finding of Kumar and Saini, (2011) and Abouelnasr *et al*. (2012). In our study reticular wall was detected in the thoracic cavity "beneath lung" as in (Fig. 3a and 3b) and "beneath heart compressing the left ventricle' as in (Fig. 3c and 3d). In this investigation, reticular movement in the thoracic cavity was reduced or completely absent and this restricted movement may be attributed to herniation or adhesions (Kumar *et al*., 2007), although Athar *et al*. (2010) recorded a complete reticular motility in the thoracic cavity in animals with DH. So the ultrasonography is a better technique for diagnosis of diaphragmatic hernia in buffaloes as compared with other diagnostic methods (Neeraj *et al*., 2013).

Conclusion

In summary, DH is a common problem in buffaloes compared with other ruminant species, thorough medical evaluation is needed before initiating surgical operation. Clinical signs and hemato-biochemical findings are of great values; however, additional diagnostic aids are essential. Ultrasonography may be an accurate tool in the diagnosis of DH.

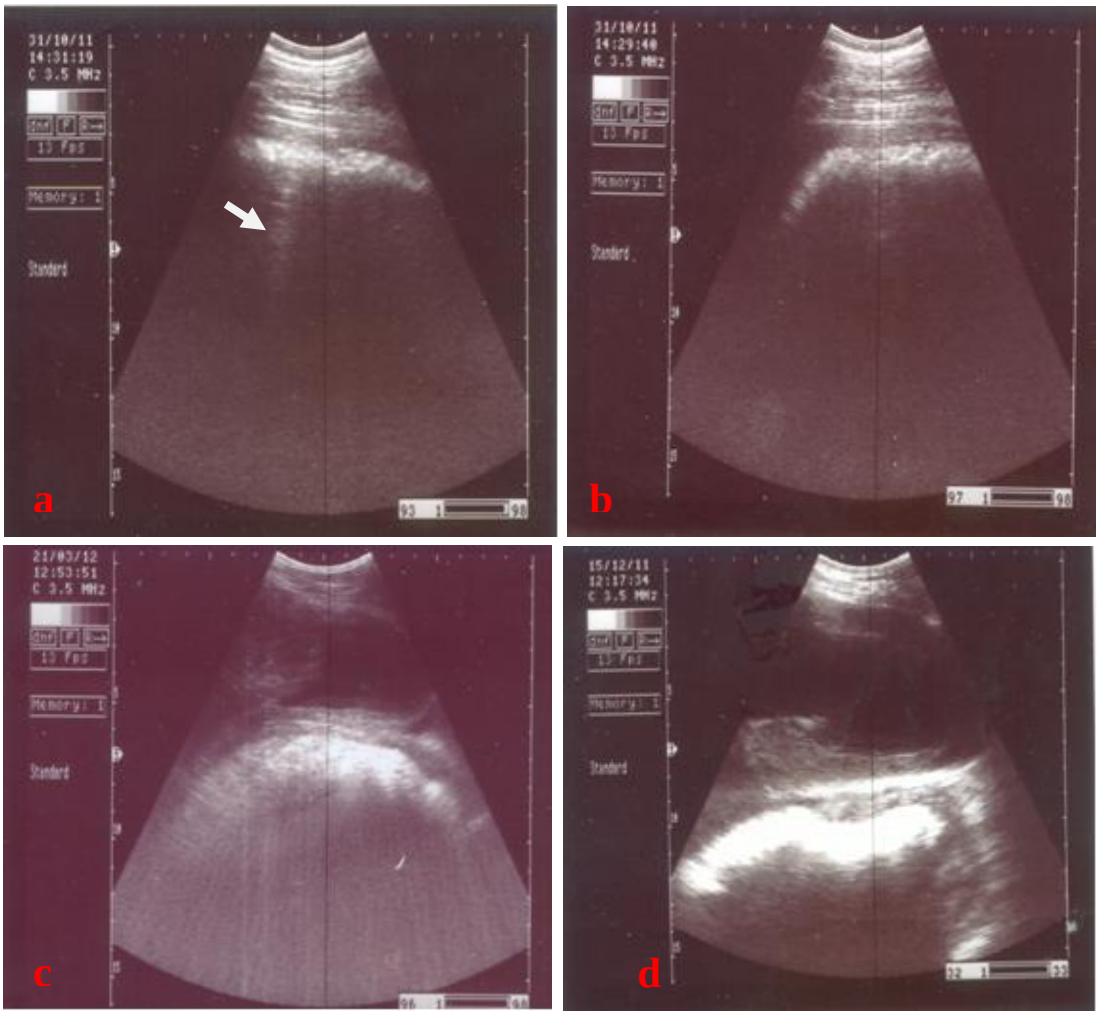

Fig. 3: Reticulum at thoracic cavity (lung and heart) imaged from left 5th ICS beneath lung (a&b). Notice reverberation artifacts "white arrow', and 4th ICS beneath heart (c&d). Herniated reticulum appears half-moon shape and undulating.

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