Research Article

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

Noura EA Attia

Animal Medicine Department, Faculty of Veterinary Medicine, Zagazig University, Egypt

*Corresponding author: noura_abobaker@yahoo.com

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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 4th/5th 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, Ultrasonography, 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
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 Hordagoda 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 unhampered 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 5th 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
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, antialoat agents and stomachics.

The animals showed congested mucous membrane and engorgement of sceral 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 viscer plays 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 leukocytes 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:

<table>
<thead>
<tr>
<th>Clinical findings</th>
<th>Control group</th>
<th>DH group</th>
<th>Abnormal findings</th>
<th>No. of animal</th>
<th>Percent %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appetite</td>
<td>Good</td>
<td>Reduced- anorexia</td>
<td></td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>Recurrent tympany</td>
<td>Negative</td>
<td>Positive</td>
<td></td>
<td>5</td>
<td>41.66</td>
</tr>
<tr>
<td>Persistent tympany</td>
<td>Negative</td>
<td>Positive</td>
<td></td>
<td>7</td>
<td>58.33</td>
</tr>
<tr>
<td>Feces</td>
<td>Semi-solid</td>
<td>Scanty hard</td>
<td></td>
<td>8</td>
<td>66.66</td>
</tr>
<tr>
<td>Regurgitation of food from mouth</td>
<td>Negative</td>
<td>Scanty soft</td>
<td></td>
<td>2</td>
<td>16.66</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>Negative</td>
<td>Diarrhoea</td>
<td></td>
<td>2</td>
<td>16.66</td>
</tr>
<tr>
<td>Pain test</td>
<td>Negative</td>
<td>Diastic</td>
<td></td>
<td>9</td>
<td>75</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>Negative</td>
<td>Dyspneic</td>
<td></td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>Pain test</td>
<td>Negative</td>
<td>Positive</td>
<td></td>
<td>9</td>
<td>75</td>
</tr>
<tr>
<td>Ruminal movement/ 2minutes</td>
<td>3.00±0.3</td>
<td>Decreased '0.7±0.2’</td>
<td></td>
<td>7</td>
<td>58.33</td>
</tr>
<tr>
<td>Abnormal lung sound</td>
<td>Negative</td>
<td>Stasis</td>
<td></td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>Systolic heart sound</td>
<td>Negative</td>
<td>Increased '6-7/2min’</td>
<td></td>
<td>2</td>
<td>16.66</td>
</tr>
<tr>
<td>Respiration rate/minute.</td>
<td>24.8±3.7</td>
<td>36.4±1.0</td>
<td></td>
<td>36.4±1.0</td>
<td>0.000</td>
</tr>
<tr>
<td>Rectal temperature</td>
<td>38.48±0.03</td>
<td>38.34±0.13</td>
<td>0.351</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate/minute</td>
<td>66.6±1.12</td>
<td>43.20±4.22</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*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 hematoo-biochemical findings are of great values; however, additional diagnostic aids are essential. Ultrasonography may be an accurate tool in the diagnosis of DH.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group (n=10)</th>
<th>DH group (n=12)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEC 10⁶/ml</td>
<td>6.8±0.18</td>
<td>5.1±0.1</td>
<td>0.000</td>
</tr>
<tr>
<td>TLC10⁶/ml</td>
<td>6.6±0.4</td>
<td>13.16±1.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Hb. g/dl</td>
<td>11.26±0.24</td>
<td>8.18±0.13</td>
<td>0.000</td>
</tr>
<tr>
<td>PCV%</td>
<td>30.1±0.65</td>
<td>35.8±1.53</td>
<td>0.009</td>
</tr>
<tr>
<td>Glucose mg/dl</td>
<td>60.6±1.4</td>
<td>42.4±1.4</td>
<td>0.000</td>
</tr>
<tr>
<td>Total proteins g/l</td>
<td>6.4±0.2</td>
<td>7.5±12</td>
<td>0.010</td>
</tr>
<tr>
<td>Albumin g/l</td>
<td>3.8±0.2</td>
<td>2.7±0.08</td>
<td>0.003</td>
</tr>
<tr>
<td>Globulin g/l</td>
<td>2.6±0.15</td>
<td>4.8±0.94</td>
<td>0.000</td>
</tr>
<tr>
<td>Hp g/l</td>
<td>0.03±0.01</td>
<td>1.14±0.1</td>
<td>0.000</td>
</tr>
<tr>
<td>SAA ug/ml</td>
<td>65.0±1.7</td>
<td>163.20±2.817</td>
<td>0.000</td>
</tr>
<tr>
<td>Fb mg/dl</td>
<td>246.8±21.8</td>
<td>605.8±44.2</td>
<td>0.000</td>
</tr>
<tr>
<td>AST iu/l</td>
<td>74.2±1.68</td>
<td>159.0±6.55</td>
<td>0.000</td>
</tr>
<tr>
<td>ALT iu/l</td>
<td>32.80±1.43</td>
<td>65.0±2.92</td>
<td>0.000</td>
</tr>
</tbody>
</table>

* P is significant at <0.05
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.

REFERENCES


