Assessment of pulmonary function test, acute phase proteins, cytokines and electrocardiographic changes in naturally occurring bovine respiratory disease of feedlot cattle calves

Mohamed A. Ramadan1, Yassein M. Abd El-Raof2, Mohamed M. Zeineldin1, Hussam E. M. El-attar1, Abdelghany H. abdelghany2, Mohamed M. Ghanem1

1Animal Medicine Department, Faculty of Veterinary Medicine, Benha University, Egypt
2Animal Medicine Department, Faculty of Veterinary Medicine, Menoufiya University, Egypt

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ABSTRACT

The aim of this study was to evaluate pulmonary function test, acute phase proteins, pro-inflammatory cytokines, electrolyte balance and electrocardiographic alterations in bovine respiratory diseases (BRD) affected calves compared to pen matched healthy control calves. A total of 30 calves (20 BRD affected calves and 10 apparently healthy calves) were used in this study. BRD affected calves showed significant (P<0.05) reduction in blood pH, partial pressure of oxygen, oxygen saturation, calcium, phosphorus, magnesium, sodium and chloride, and significant (P<0.05) increase in partial pressure of carbon dioxide, total carbon dioxide and potassium. Acute phase proteins and proinflammatory cytokines assessment revealed a significant (P<0.05) increase of serum amyloid A, haptoglobin, fibrinogen and interleukin-6 in diseased calves compared to healthy control calves. Electrocardiographic examination of BRD affected calves revealed a significant increase in heart rate with a significant (P<0.05) alteration in electrocardiographic changes (ECG) wave trace parameters (P wave, QRS complex, T wave, PR interval, QT interval). In conclusion, measurement of pulmonary function test, APPs, cytokines and ECG could be used as valuable and early diagnostic tools for BRD diagnosis in feedlot cattle calves.

1. INTRODUCTION

Bovine respiratory disease (BRD) is one of the most economically significant diseases in cattle industry especially in intensely raised, recently weaned and newly transported calves (Loneragan et al., 2001). The biggest challenges in bovine medicine is an early detection of clinical cases of diseases, especially important for subclinical form, which can be easily missed and cause important economic losses (Arslan and Ozcan, 2018). It has been reported that 37–68% of calves that never received treatment for BRD during the finishing period had lung lesions at the slaughter time (Thompson et al., 2006). For this reason, early identifying of an accurate method to classify calves that are at greater risk of becoming sick is the key for optimal calves’ health (Abdallah et al., 2016; Zeineldin et al., 2019).

Recently, the possibility to use acute phase proteins (APPs), cytokines, pulmonary function test and electrocardiography (ECG) as diagnostic biomarkers of infection has expanded significantly in the context of respiratory medicine. The acute inflammatory process initiates the acute phase reaction that results in increase the concentration of APPs in diseased calves (Heller and Johns, 2015). APPs have been recently proposed as sensitive and rapid indicators of inflammatory processes in ruminants (Gonzales et al., 2011). APPs play an important role in eliminating the infectious agents and activating the repair process toward the normal function (Tothova et al., 2015). Assessment of blood gases and related clinical parameters are also considered an important diagnostic indicators for BRD that help in early treatment decision (Constable et al., 2017). ECG assessment is also a useful tool in accurate diagnosis and evaluation of cardiac diseases or dysfunctions secondary to other systemic disease (Ghanem, 1997). Therefore, our hypothesis was that assessment of pulmonary function test, APPs and ECG changes in BRD affected calves with other clinical variables could be used to influence treatment decisions at the time of initial disease diagnosis. The objective of this study was to evaluate pulmonary function test, APPs, electrolyte balance and ECG alterations in BRD affected calves compared to pen matched healthy control calves.

2. MATERIAL AND METHODS

2.1. Animals and samples collection

This study was carried out in a farm in Gharbiya Governorate. A total of 30 calves (3-9 months old) were selected and used in this study. These calves were recently transported to the farm from sale barns before beginning this study. All calves were classified based on clinical examination into the following groups: clinically healthy
calves (Control, n=10), and calves suffered from bovine respiratory disease (BRD affected calves, n=20). Calves suspected to be suffering from BRD were visually examined for the presence of nasal or ocular discharge, respiratory distress, cough, depression and inappetence. When two or more of these clinical signs were observed, rectal temperature of calf was recorded. Using the clinical scoring system (McGuirk, 2008), a calf with a score of 5 or more was classified as morbid and included in the study. Calves were not included in the study if there was a presence of concurrent diseases. All calves were subjected to complete clinical examination including body temperature, pulse rate, respiratory rate and thoracic auscultation (Radostitis et al., 2000).

Two blood samples were collected from each calf using jugular vein puncture. The first blood sample (2 ml) was collected in syringe containing hepargin (50 IU/ml) used for blood gases and plasma fibrinogen estimation (Farah et al., 2017). The second blood sample was collected without anticoagulant, clotted at room temperature for 20 min, centrifuged at 3000 rpm for 10 min, and the clear non-hemolyzed serum samples were separated and stored at -20 °C until subsequent biochemical analysis.

2.2. Blood gas analysis
Immediately after vein-puncture, the tip of the needle was sealed with a rubber stopper in order to prevent gas from moving in or out. The samples were placed in a bed of crushed ice, taken immediately to the laboratory for analysis no more than one hour after collection. The blood samples were analyzed for pH, partial oxygen pressure (PO2), partial dioxide pressure (PCO2), bicarbonate (HCO3) concentrations, total carbon dioxide (tCO2) and oxygen saturation (SO2) using blood gas analyzer (Hussein and Aamer, 2013).

2.3. Acute phase proteins and proinflammatory cytokines measurement
Serum haptoglobin (Hp) concentration was determined by ELISA kit according to method described by Idoate et al. (2015). Serum amyloid A (SAA) concentration was measured with a commercially available ELISA kit according to method described by Alsemgeest et al. (1994). Serum fibrinogen (Fb) concentration was measured according to method described by Becker et al. (1984). Interleukin-6 (IL-6) level was determined from undiluted serum samples using commercially available ELISA kits according to method described by Kabu et al. (2016a).

2.4. Serum minerals and electrolytes measurement
Serum Ca, inorganic P, Cl, Na and K levels were determined using spectrophotometer according to the method described by Chessborough (1991). Serum magnesium (Mg) levels were determined by using atomic absorption spectrophotometer by as described by Devlin (1997).

2.5. Electrocardiographic examination
The electrocardiogram (ECG) was recorded with base apex lead system II using limb lead. Calves were kept in standing position without any tranquilizers or sedative. When animals got calm (decreasing muscle tremors), ECGs were recorded, using alligator-type electrodes attached to the skin after cleaning it with ethanol and applying ethyl alcohol to improve the contact. Base apex lead system II was applied as; the right forelimb electrode was placed on the right side of the neck along the jugular groove one third of the way up the neck. The left forelimb electrode was placed on the ventral midline under the apex of the heart. The ground cables were placed on the left and right stifle joints. Alligator clips moisten with alcohol were used (Ghanem, 1997). ECG should reveal a distinct P wave (atrial depolarization), QRS complex (ventricular depolarization), and T wave (ventricular repolarization). All ECGs were obtained with a single channel electrocardiographic machine (BTL-08 SD ECG, Industries Ltd.161 Cleveland Way, Steven age, SG1 6BU, UK) with paper speed of 25 mm/s and calibration of 10 mm equal to 1 mV. For measuring ECG parameters, the ECG was analyzed using a magnifying glass. In this method, precision of duration is 0.02 sec. and amplitude is 0.1 mV.

2.6. Statistical analysis
Statistical analysis was performed using IBM SPSS Statistics version 20 (IBM Armonk, NY, USA). The data was statistically analyzed using independent sample t-test was performed to compare control healthy with diseased animal as previously described by Bailey (2008). Values were represented as means ± standard error. Differences were considered statistically significantly when P< 0.05.

3. RESULTS

3.1. Clinical signs
Calves suffered from BRD developed varying degree of depression, shallow rapid respiration, anorexia, loss of body weight, nasal discharge, sever dyspnea with mouth breathing, congestion of ocular mucous membrane with ocular discharge and some BRD cases suffered from painful cough.

Auscultation of the lung in BRD affected calves showed abnormal lung sounds including loud wheezing, crackling sound and moist rales. Fractional sound and exaggerated vesicular sounds were also heard.

BRD affected calves also showed significant (P< 0.05) increase in body temperature (40.62 ± 0.09), respiratory rate (54.25 ± 2.75) and pulse rate (154.05 ± 2.66) compared to clinically healthy calves body temperature (38.94±0.11), respiratory rate (29.1 ± 1.15) and pulse rate (93.3 ± 1.71).

3.2. Blood gas analysis
The BRD affected calves showed significant (P< 0.05) decrease in pH, PO2 and SO2, and increase in PCO2, HCO3, and tCO2, when compared to healthy control calves (Table 1).

3.3. Acute phase proteins and proinflammatory cytokines
BRD affected calves showed a significant (p< 0.05) increase in Hp, SAA, Fb and IL-6 levels when compared to healthy calves (Table 2).

3.3. Serum minerals and electrolytes
BRD affected calves showed a significant (P< 0.05) decrease in Ca, P, Mg, Na and Cl when compared to healthy calves. On other hand, diseased calves exhibited a significant (P< 0.05) increase in K level when compared to healthy calves (Table 3).

3.4. Electrocardiographic findings
In healthy control calves, heart rhythm was normal in all calves (Fig. 1). While, in BRD affected calves heart rhythm
showed some arrhythmias such as sinus tachycardia, sinus arrhythmia, and atrial fibrillation (Fig. 2-4). In comparison to control calves, the BRD calves showed significant (P<0.05) increase in QRS amplitude, and T wave duration and amplitude. Also, there was significant (P<0.05) decrease in P wave duration and amplitude, QRS duration, P-R interval and Q-t interval when com control group (Table 4).

Table 1 Blood gas analysis of apparently healthy calves and BRD affected calves

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=10)</th>
<th>BRD affected calves (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.34±0.006a</td>
<td>7.31±0.012b</td>
</tr>
<tr>
<td>HCO3 (mEq/L)</td>
<td>25.7±1.72a</td>
<td>28.1±1.46b</td>
</tr>
<tr>
<td>PCO2 (mmHg)</td>
<td>40.0±6.98a</td>
<td>56.1±1.07a</td>
</tr>
<tr>
<td>PO2 (mmHg)</td>
<td>33.5±1.61a</td>
<td>25.76±1.62b</td>
</tr>
<tr>
<td>iCa (mEq/L)</td>
<td>26.9±1.68a</td>
<td>30.49±1.55b</td>
</tr>
<tr>
<td>SO2 (%)</td>
<td>64.6±3.01a</td>
<td>47.23±1.56b</td>
</tr>
</tbody>
</table>

Data represented as Mean ± SE. Superscript letters indicated significance difference between groups on P<0.05.

Table 2 Serum acute phase proteins and pro-inflammatory cytokines of apparently healthy and BRD affected calves

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=10)</th>
<th>BRD affected calves (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAA (mg/L)</td>
<td>20.7±2.10a</td>
<td>123.23±5.18b</td>
</tr>
<tr>
<td>Hp (mg/dL)</td>
<td>1.17±0.11a</td>
<td>6.69±0.26a</td>
</tr>
<tr>
<td>Fb (g/L)</td>
<td>3.79±0.24a</td>
<td>9.79±0.75b</td>
</tr>
<tr>
<td>IL-6 (ng/mL)</td>
<td>94.5±4.16a</td>
<td>276.42±5.08b</td>
</tr>
</tbody>
</table>

Data represented as Mean ± SE. Superscript letters indicated significance difference between groups on P<0.05.

Table 3 Serum mineral and electrolyte level of apparently healthy and BRD affected calves

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=10)</th>
<th>BRD affected calves (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K+ (mEq/L)</td>
<td>3.5±0.23a</td>
<td>3.12±0.32b</td>
</tr>
<tr>
<td>P (mEq/L)</td>
<td>6.1±0.42a</td>
<td>4.5±0.23a</td>
</tr>
<tr>
<td>Mg (mEq/L)</td>
<td>0.93±0.023a</td>
<td>0.77±0.019a</td>
</tr>
<tr>
<td>Na (mEq/L)</td>
<td>140.6±4.15a</td>
<td>130.72±1.04a</td>
</tr>
<tr>
<td>K (mEq/L)</td>
<td>4.3±0.11a</td>
<td>6.26±0.18a</td>
</tr>
<tr>
<td>Cl (mEq/L)</td>
<td>106.8±4.37a</td>
<td>88.1±1.23a</td>
</tr>
</tbody>
</table>

Data represented as Mean ± SE. Superscript letters indicated significance difference between groups on P<0.05.

4. DISCUSSION

The major clinical signs of BRD calves were shallow rapid respiration indicating hypoxia due to dyspnea that might be attributed to severe inflammation in the bronchioles and alveoli which interfere with gas exchange and respiration. In agreement with other studies, BRD affected calves showed a significant increase in PCO2, iCO2 and HCO3 and a significant decrease in pH, PO2 and SO2 when compared to control group (Nagy et al., 2006; Fararh et al., 2017). The alteration of blood gas analysis may be attributed to the pathological actions of microorganisms on lung which lead to alteration in blood gas exchange and blood pH (Karademir et al., 1999). The increase in HCO3 level may be also attributed to response of kidney to the respiratory acidosis through renal retention of bicarbonate in exchange with chloride to maintain electrical neutrality (Kumar et al., 2018). The increase in PCO2, HCO3 and iCO2 and decrease in PO2 level may be attributed to a high respiratory frequency and aerobic metabolism to reduce the level of CO2 (Nagy et al., 2006; Smith, 2014). Decrease PO2 and SO2 besides the disturbance in gas exchanges might be attributed to the consumption of oxygen during metabolism that resulted from elevated WBCs count (Hussein and Aamer, 2013).

Serum APPs and proinflammatory cytokines of BRD calves revealed a significant increase of SAA, Hp, Fb and IL-6 which agreed with Fararh et al. (2017), Joshi et al. (2018) and Akgul et al. (2019). The proinflammatory cytokines are proteins that act as chemical messengers secreted by T-cells in response to the host immune responses (Ditchkoff et al., 2001). Colonization of respiratory pathogens causes release of proinflammatory cytokine and transcription of many cytokine genes particularly IL-6 (Ridpath, 2010). Development of BRD is also associated with complex signaling processes that play a crucial role in recognizing the
microbial components by innate immune cells followed by phagocytosis of invading pathogens and production of IL-6 and other pro-inflammatory cytokines (Ackermann et al., 2010). Cytokines play an important role in elimination of the infection through modulation of other immune proteins (APPs) or stimulation of phagocytosis (Tothova et al., 2008). APPs are produced by both hepatocytes and peripheral tissues in response to inflammatory process (Ceciliani et al., 2012). Acute phase proteins have been proposed as sensitive and rapid indicators of inflammatory processes in ruminants (Gonzales et al., 2011). Increased Hp, SAA, Fb level might be a consequence of severe lung tissue injury as a result of the inflammation in BRD affected calves which highlight their role in host immunity (Orro et al., 2011). The rates of increment of Hp and SAA were different in BRD, while Hp increased immediately after infection, SAA took relatively longer time. These findings might be associated with different induction rates of APPs in liver (Joshi et al., 2018). In our study, SAA and Hp increased in all BRD affected calves with relatively similar level, which indicated that both SAA and Hp are highly sensitive to occurrences of BRD in calves. Fibrinogen was also highly increased in calves with BRD (Akgul et al., 2019). From this study, SAA had the highest increased value followed by Hp and fibrinogen. These findings suggest that Hp and SAA were the best markers for detection of naturally occurring respiratory disease in cattle (Abdelbaset et al., 2014).

The serum minerals and electrolyte levels of BRD calves revealed significant (P<0.05) decrease of Ca, P, Mg, Na and Cl when compared to control group. On other hand, BRD affected calves showed significant (P<0.05) increase in K when compared to control calves. This result is in agreement with Ismael et al. (2017). Inappetence or anorexia as a result of respiratory disease might explain the lower plasma concentrations of measured minerals (Kumar et al., 2018). The decreased in serum Ca might be resulted from the anorexia, decreased intestinal absorption or increased renal excretion and the significant decrease in serum P concentrations seemed to be secondary to reduced phosphorus absorption from the gut and tissues (Saleh and Allam, 2014). Higher K concentrations in serum could be seen as well in BRD cases because H+ ions accumulated in the extracellular fluid is exchanging with K present in the intracellular fluid (Kaneko et al., 2008). Reduction of serum Cl and increased K level might be also attributed to hyperpyrexia in acute respiratory disease and metastatic infection of kidneys (Constable et al., 2017). ECG findings of BRD affected calves in this study showed a significant decrease in P-wave amplitude and duration (reduced atrial contractility or depolarization), which agreed with Devi et al. (2015). This could be attributed to atrial fibrillation or supraventricular pacemaker fibrillation as a result of myocardial hypoxia (Gelberg et al., 1991; Reef and McGuirk, 1996). This also could be resulted from the alteration in hemodynamic/acid-base balance or hypoxic changes as a result of respiratory distress (Smith, 2014; Devi et al., 2015). Reduced atrium contractility (depolarization) might be resulted from the hypocalcemia and the significant decrease in QRS-wave duration resulted from increased heart rate with interference with pulmonary circulation and agreed with Gelberg et al. (1991), Reef and McGuirk (1996) and Devi et al. (2015). On the other hand, the significant increase in T-wave amplitude, with a significant decrease in P-R interval and Q-T interval might be attributed to disturbance in ventricular relaxation or ventricular hypertrophy (Smith, 2014; Devi et al., 2015). Moreover, the electrolyte imbalance (hyperkalemia) resulted in increase in the peaking of T wave (Johri et al., 2009).

5. CONCLUSIONS

In conclusion, our study showed that the BRD was associated with changes in pulmonary function, APPs, proinflammatory cytokines, electrolyte balance and electrocardiographic traces. Pulmonary function test, APPs and proinflammatory cytokines are valuable tools in early diagnosis of BRD so they should be included in at the time of initial diagnosis of BRD.

6. REFERENCES


