# Evaluation of clinical and hematobiochemical alterations in naturally occurring bovine respiratory disease in feedlot cattle calves in Egypt.

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## A B S T R A C T

The purpose of this study was to evaluate the changes in the clinical and hemato-biochemical parameters of cattle calves suffering from bovine respiratory disease (BRD) compared to pen matched healthy control calves. This study was conducted on 20 calves suffered from BRD and 10 apparently healthy calves. Clinical and physical examination of BRD affected calves revealed significant ($P<0.05$) increase in pulse rate, body temperature, respiratory rate, anorexia, nasal discharge, coughing, dyspnoea and abnormal lung sounds. Hematological analysis showed that the BRD affected-calves revealed significant ($P<0.05$) decrease in red blood cells (RBCs) count, haemoglobin (Hb) content, packed cell volume (PCV %) and lymphocytes count, with significant ($P<0.05$) increase in white blood cells (WBCs), neutrophils, monocytes and eosinophils. Biochemical analysis showed significant ($P<0.05$) increase in total proteins, globulin, with significant ($P<0.05$) decrease in albumin and albumin/globulin (A/G) ratio in diseased calves compared to healthy control. Moreover, the BRD affected calves showed significant ($P<0.05$) increase in liver enzymes activity and kidney function test. In conclusion, the results of this study revealed that the respiratory diseases in calves have severe impacts on several hematological and biochemical parameters.

**Key words:** *BRD, Calves, Clinical, Hematobiochemical*.

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## 1. INTRODUCTION

Bovine respiratory disease (BRD) is a general term for respiratory disease in cattle and caused by a wide range of factors, singly or in combination including; bacterial pathogens, viral agents, and various environmental conditions (Nickell and White, 2010). BRD is the most common and costly disease worldwide in both dairy and feedlot cattle (Edwards, 2010 and Zeineldin *et al.,* 2019). Bovine respiratory disease is also one of the most major causes of morbidity and mortality with severe economic losses in cattle industry due to loss of production, labor, increased time on feed, prophylaxis, and metaphylaxis treatments (Abutarbush *et al.*, 2012). BRD affected calves exhibiting signs of fever, nasal discharge, depression, cough, nasal discharges, abnormal lung sound, separate themselves from the rest of the pen along with sever alteration in blood gases (Ozkanlar, 2012 and Zeineldin *et al.,* 2017a). This generally occurs within the first two weeks after transported to the feedlot farm (Zeineldin *et al.*, 2017b). Hematological picture of BRD-affected calves characterized by changes in blood Hb, RBCs, PCV, total and differential leucocytic count (Kumar *et al*. 2018). Serum biochemical alterations in BRD-affected calves were also common and might display reasonably predictable changes in response to inflammation and resulted in significant difference in protein profile (El-Seidy *et al.,* 2003 and Šoltésová *et al.,* 2015). The previous published studies revealed that the alterations in the biochemical parameters (e.g. liver enzyme activity level, urea and creatinine level) during the course of respiratory disease resulted from the hyperpyrexia and metastatic infection during the course of BRD (Novert, 2004 and Metwally *et al.,* 2017). The present study was designed to record the clinical picture of naturally occurring respiratory disease and to evaluate the haemato-biochemical alterations in BRD affected calves.

## 2. MATERIALS 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 are recently transported to the farm from sale barns within one to two weeks ago before begin this study. All calves were classified based on clinical examination into the following groups: clinically healthy calves (Control, n=10), and calves suffered from 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 inappetance. When 2 or more of these clinical signs were observed, rectal temperature of calf was recorded. Using the clinical scoring system (McGuirk, 2008) summarized in Table 1, 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 (Radostits *et al.,* 2000). Two blood samples were collected from each calf using jugular vein puncture (Radostits *et al.,* 2000). The first blood sample was collected on labeled test tube containing 5 mg $k_2$EDTA in concentration of 1 mg/1ml blood (Coles, 1986) as an anticoagulant for determination of hematological parameters (RBCs count, Hb content and PCV %). The second blood sample was collected without anticoagulant, clotted at room temperature for 20 min, centrifuged at 3,000 rpm for 10 min, and then the clear non-hemolyzed serum samples were separated and stored at −20°C until subsequent biochemical analysis.

### *2.2. Hematological and Biochemical analysis*

Total erythrocytic count (RBCs), Hemoglobin concentration (Hb), packed cell volume (PCV), Total leukocytic count (WBCs) and differential leukocytic counts were determined by hematological analyzer as previously described by Feldman *et al.* (2000). Serum total proteins were determined spectrophotometrically according to the method described by Pagana and Pagana (2010). Albumin was determined calorimetrically by using the dye-binding technique with bromocresol green and A/G ratio was calculated by dividing the albumin value over globulin value according to Fischbach and Dunning (2009). Serum globulin was determined by the differences between total protein and albumin according to Chernecky and Berger (2008). Serum samples were used also for determination of Aspartate transaminase (AST), Alanine aminotransferase (ALT), Creatine kinase (CK) and Lactate dehydrogenase (LDH) using of the special kits according to the method that described by Pagana and Pagana, (2010); Thefeld (1974) and Hare (1950) respectively. Serum urea and creatinine concentration were also determined spectrophotometrically using special kits according to the method described by Walker *et al.* (1990); Peake and Whiting (2006) respectively.

### *2.3. 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 healthy with diseased animal as previously described by Bailey (2008).Values were represented as means ± standard error (SE).

All differences were considered statistically significantly when $P<0.05$.

## 3. RESULTS

### *3.1. Clinical findings and physical examination*

Clinical examinations of calves suffered from BRD are diagnosed based on McGuirk's clinical scoring system. The most common recognizable clinical signs of those calves that develop BRD were fever, varying degree of depression, shallow rapid respiration, in-appetence, off feed, nasal discharge (Figure, 1 and 2), sever dyspnea with mouth breathing in some cases (Figure, 3), congestion of ocular mucous membrane with ocular discharge (Figure, 4), loss of body weight and some BRD cases suffered from painful cough. Auscultation of the lung in BRD affected calves showed miscellaneous lung sounds including loud wheezing, crackling sound and moist rales. Frictional sound and exaggerated vesicular sounds were also heard. BRD affected calves also showed significant increase in body temperature, respiratory rate and pulse rate compared to clinically healthy calves (Table 2). Clinical examination of apparently healthy calves (control group) revealed good healthy condition with no apparent injury or diseases. The healthy control calves showed normal behavior during handling and examination, shiny hair, healthy eyes, moist muzzle with no ocular and nasal discharge. The body temperature, pulse and respiration of clinically healthy calves were within the normal range (Table 2).

### *3.2. Hematological findings*

Table (3) showed that the RBCs count, Hb content and PCV% were significantly ($P<0.05$) decreased in BRD affected calves compared to healthy control calves. BRD affected calves also showed a significant ($P<0.05$) increase in WBCs, neutrophil, monocytes, and eosinophils count with significant ($P<0.05$) decrease in lymphocytes count when compared to healthy control calves.

### *3.3. Biochemical findings*

Table (4) showed that blood proteins analysis in BRD affected calves revealed a significant ($P<0.05$) increase of total proteins and globulin with significant ($P<0.05$) decrease in albumin and A/G ratio when compared to clinically healthy calves. Assessment of serum enzymes and kidney function in BRD affected calves showed a significant ($P<0.05$) increase in ALT, AST, CK, LDH, Urea and Creatinine when compared to clinically healthy calves.

Table (1). McGuirk's clinical scoring system for the screening of calves suffering from BRD.

| Score / Parameter | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| Rectal temperature(°C) | 37.7–38.2 | 38.3–38.8 | 38.9–39.3 | ≥39.4 |
| Cough | None | Induced single cough | Induced repeated coughs or occasional spontaneous cough | Repeated spontaneous coughs |
| Nasal discharge | Normal serous discharge | Small amount of unilateral cloudy discharge | Bilateral cloudy or excessive mucus discharge | Copious bilateral mucopurulent discharge |
| Eye score | Normal | Small amount of ocular discharge | Moderate amount of bilateral discharge | Heavy ocular discharge |
| Ear score | Normal | Ear flick or head shake | Slight unilateral droop | Head tilt or bilateral droop |

A calf with a score of 5 or more was classified as BRD affected calf

Table (2): Physical examination of apparently healthy and BRD affected calves

| Parameters Groups | Control calves (n=10) | BRD calves (n=20) |
|---|---|---|
| Temperature (c°) | 38.94±0.11 [a] | 40.615±0.09 [b] |
| Pulse rate (beat /min) | 93.3±1.71 [a] | 154.05±2.66 [b] |
| Respiration rate (breath/min) | 29.1±1.15 [a] | 54.25±2.75 [b] |

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

Table (3): Hematological parameters of apparently health and BRD affected calves.

| Parameters Groups | Control calves (n=10) | BRD calves (n=20) |
|---|---|---|
| RBCs *($10^6/\mu l$)* | 6.19±0.71 [a] | 4.91±0.64 [b] |
| Hb *(gm/dl)* | 9.45±0.09 [a] | 8.23±0.88 [b] |
| PCV *(%)* | 33.21±0.69 [a] | 25.05±0.58 [b] |
| TLC*($10^3/\mu l$)* | 9.26±0.19 [a] | 13.17±0.42 [b] |
| Lymphocytes *($10^3/\mu l$)* | 4.25±0.09 [a] | 3.72±0.12 [b] |
| Neutrophils *($10^3/\mu l$)* | 4.04±0.08 [a] | 7.57±0.24 [b] |
| Monocytes*($10^3/\mu l$)* | 0.57±0.01 [a] | 1.14±0.04 [b] |
| Eosinophils ($10^3/\mu l$) | 0.29±0.006 [a] | 0.46±0.019 [b] |

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

Table (4) Serum biochemical analysis of apparently healthy and BRD affected calves.

| Parameters Groups | Control calves (n=10) | BRD calves (n=20) |
|---|---|---|
| Total Protein (g/dl) | 7.12±0.23 [a] | 7.49±0.09 [b] |
| Albumin (g/dl) | 3.34±0.095 [a] | 2.67±0.068 [b] |
| Globulin (g/dl) | 3.72±0.25 [a] | 4.82±0.13 [b] |
| A/G ratio | 0.886±0.079 [a] | 0.554±0.029 [b] |
| ALT(U/L) | 21.17±0.95 [a] | 35.95±1.01 [b] |
| AST(U/L) | 86.86±3.08 [a] | 123.33±8.08 [b] |
| CK (U/L) | 175.04±8.55 [a] | 248.51±10.4 [b] |
| LDH(U/L) | 744.1±14.1 [a] | 862.3±17.7 [b] |
| Urea(mg/dl) | 17.18±1.04 [a] | 25.66±1.42 [b] |
| Creatinine(mg/dl) | 1.16±0.054 [a] | 1.53±0.075 [b] |

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

Figure 1: BRD affected calf showing mucopurulent nasal discharge.

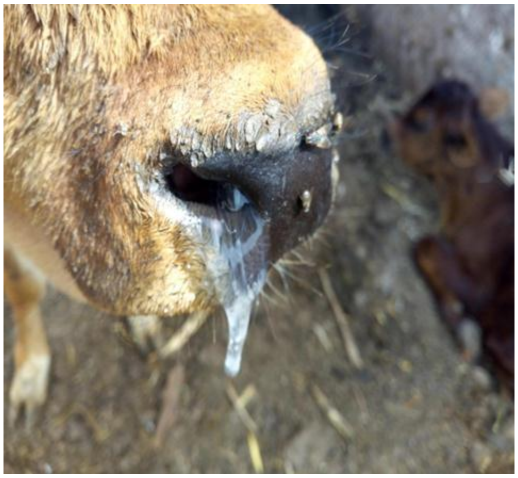

Figure 2: BRD affected calf showing mucoid nasal discharge

Figure 3: BRD calve showing mouth breathing with protrusion of tongue due to dyspnea.

Figure 4: BRD Calve showing ocular discharge

## 4. DISCUSSION

The major clinical signs of BRD affected calves were shallow rapid respiration due to hypoxia and dyspnea in some cases that might be attributed to severe inflammation in bronchioles and alveoli that interfere with gas exchange and respiration. The observed nasal discharges might be also attributed to inflammatory changes in the nasal mucus membrane. In BRD affected calves, heart rate was increased to compensate the hypoxia. In addition, some BRD cases suffered from painful cough, depression and decreased appetite. Auscultation of the lung in BRD affected calves showed miscellaneous lung sounds including loud wheezing, crackling sound, moist rales due to the exudates produced by inflammatory cells and goblet cells as a result of pneumonia. Frictional sound and exaggerated vesicular sounds were also heard. These clinical findings in diseased calves were comparable with those recorded by Ismael *et al.*, (2017); Metwally *et al.*, (2017) and Kumar *et al.*, (2018). Our study populations were previously subjected to transportation before enrollment in the study. The transportation into new environment along with mixing between old calves in the same place and exposure to different pathogens are considered crucial predisposing factors for BRD development as previously reported by Griffin *et al.*, (2010) and Zeineldin *et al.*, (2019). Commingling of calves with other cattle in sale barns is an important management factor predisposing cattle to BRD. Because sale barn cattle have greater exposure to pathogens and stress as a result of mixing with cattle from multiple sources, feedlot cattle purchased from sale barn are often at greater risk for BRD compared to the ones purchased directly from the farm or ranch (Amat, 2019).Similar to the previous study, the hematological examination (*Table, 3*) of the BRD affected calves revealed significant decrease in RBCs count, Hb (gm/dl) and PCV (%) (Ismael *et al*., 2017). These results in diseased calves might be attributed to micro-hemorrhages and sequestration of red blood cells during disseminated micro-thrombosis (Gutierrez *et al.* 1999). It could also be resulted from destruction of red blood cells by micro-organisms secretions (Mondal *et al.* 2004). Anemia could also attribute to reduction of energy and protein intake or sequestration of iron in bone marrow macrophages and hepatocytes during infection, thus become unavailable for utilization in hemoglobin synthesis leading to inhibition of erythropoiesis (Aytekin *et al.* 2011). In BRD affected calves and compared to control calves, there was a significant increase in both WBCs count, neutrophils and monocytes but there was a significant decrease in lymphocytes. These results agreed with Ismael *et al.,* (2017) and Kumar *et al*., (2018). The increase in WBC count, mainly neutrophils, is a frequent finding in many diseases as a consequence of inflammatory processes like in respiratory diseases. The high concentration endotoxin can cause lysis of lymphocytes leading to a significant lymphopenia in calves suffering from respiratory infection (Kumar *et al*., 2018 and Akgul *et al*., 2019). The significant increase in WBCs and neutrophils occurred in pneumonia might be attributed to inflammatory lesions and presence of bacterial infection (Sayed *et al.*, 2002 and Smith, 2014). On the other hand, the significant decrease of lymphocytes might be attributed to stimulation of adrenal gland with the tissue invaded by bacterial toxins or might be resulted from immune suppression as a result of various stress conditions (El-Naser and Khamis, 2009 and Aytekin *et al.*, 2011).

Serum proteins analysis of BRD affected calves revealed a significant increase of total proteins and globulin and significant decrease in albumin and A/G ratio (*Table 4)*. These results agreed with Csilla *et al.*, (2013); Metwally *et al.*, (2017) and Kumar *et al.*, (2018). Alteration in protein profile corresponds to changes occurs during acute phase response. High level of total protein in blood of the diseased calves and increase the production of immunoglobulin are usually associated with the inflammatory processes. Hyperproteinemia is usually related to infection and inflammation, due to increased synthesis of acute phase proteins, complement proteins and immunoglobulins. The significant decrease in A/G values occurs mainly due to the increased immunoglobulin synthesis following antigenic stimulation (Evan *et al.*, 2003 and El-Seidy *et al.*, 2003). Serum albumin is the major negative acute phase protein. During the acute phase response, the request for amino acids for production of the positive acute phase proteins is significantly increased, which necessitates reprioritization of hepatic protein synthesis. It has been reported that during the acute phase response 30–40% of hepatic protein anabolic capacity is used for the production of positive acute phase proteins; thus, the production of other proteins needs to be curtailed which result hypoalbuminemia (Šoltésová *et al.*, 2015). The significant increase in globulin in BRD affected calves in our study might be due to the stimulation of immune system as a result of the infectious agents (Abd El-Raof and Hassan, 1999).

Similar to the previous reports, the result of serum enzymes and kidney function assessment in BRD affected calves showed significant ($P$<0.05) increase in ALT, AST, CK, LDH, Urea and Creatinine when compared to healthy control calves (Almujalli *et al.*, 2015; Metwally *et al.*, 2017 and Kumar *et al.*, 2018). Higher activity of AST and LDH in calves with BRD probably resulted from increased respiratory rate and muscle work during prolonged duration or severe cases of respiratory disease (Šoltésová *et al.*, 2015). There was also significant increase in the levels of ALT, AST, and CK in diseased calves when compared to healthy control calves which might be allied with possible hepatic dysfunction persuaded by inflammatory response in pneumonic calves (Almujalli *et al.*, 2015). The increase in serum urea concentration of BRD calves could be explained by the accelerated catabolism of body protein and could result as a response to infection while the increase in serum creatinine due to kidney dysfunction after infection (Constable et *al.,* 2017).

## 5. CONCLUSION

BRD is one of the most economically significant diseases in feedlots cattle calves. Transportation and mixing of animals in sale barns is an important predisposing factor of BRD. Clinical and hemato-biochemical parameters can be used as a prognostic tool of the healthy condition of BRD affected calves.

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