

## Clinical, Biochemical and Bacteriological Investigation of Pneumonia in Calves with Special Reference to Alpha-1-Acid Glycoprotein Response

Research Article

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### Abstract

In order to investigate clinical and biochemical parameters in calves with bovine respiratory disease (BRD), twenty-five Holstein calves with clinical picture of BRD were selected to this investigation. Ten clinically healthy calves were selected as a control group. Blood, nasal and bronchoalveolar lavage were obtained from all calves under investigation. Complete blood parameters picture were investigated. Serum total protein, albumin, Triglyceride (TAG), High Density Lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), Total cholesterol, Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), and Alkaline Phosphatase (ALP) were examined. Alpha-1-acid glycoprotein (AGP) was estimated in all calves under investigation. Bacteriological examination showed *Pasteurella spp* in 18 calves and *Escherichia coli* in seven calves. The laboratory results revealed a significant ( $P \leq 0.05$ ) increase in the levels of white blood cells and neutrophilia in calves with pneumonia when compared with control groups. Moreover, there was a significant ( $P \leq 0.05$ ) increase in the values of TAG, VLDL-c, LDL-c, ALT, AST, ALP and AGP with significant ( $P \leq 0.05$ ) decrease in the levels of total protein, albumin, cholesterol, HDL-c in calves with BRD when compared with control ones. From the present study, it could be concluded that AGP and lipoprotein profile could be used as diagnostic markers for BRD in calves.

**Keywords:** Calves; Pneumonia; Alpha-1-acid Glycoprotein; Lipid Profile.

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### Introduction

Bovine respiratory disease (BRD) considers one of the most imperative health problem and costly disorder happening in cattle in different localities. Amplified mortality and morbidity rates, diminished feed conversion rate, reduced feed intake, reduction in the meat quality and augmented prophylaxis and therapy lead to

immense economic losses [30].

The most clinically detected signs of BRD include fever (about 40-41.5°C), misery, loss of appetite, nasal and ocular discharge, coughing and dyspnea of varying degrees. The causes of BRD is practically caused by different microorganisms (*Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, *Mycoplasma bovis*) and most commonly is linked with influencing risk factors related to host or environmental stressors [30].

The acute phase proteins (APPs) consist of proteins that display a reduction and an increase in values, in reply to any challenge. They are categorized into positive and negative APPs. The negative APPs comprise transferrin and albumin, the most copious constitutive plasma protein. The positive one is glycoproteins created mostly by hepatic cells upon stimulation by pro-inflammatory cytokines and released into blood stream. The positive APPs include, Haptoglobin, C-reactive protein, serum amyloid A, alpha-1-acid glycoprotein, fibrinogen and ceruloplasmin [8].

Little is known about the use of lipoprotein profile and Alpha-1-acid glycoprotein in cases of BRD in calves, which is the main objective of the current study.

### Materials and Methods

#### Animals

Twenty-five Holstein calves (2-4 month old) from a private farm in Harad region, Saudi Arabia with clinical picture of BRD in-

cluding fever, polypnea, nasal discharge, dyspnea, crackles on chest auscultation and loss of appetite were selected to the current investigation. In addition, ten clinically healthy calves were selected as a control group. Blood, nasal and bronchoalveolar lavage were obtained from all calves under investigation.

### Sampling protocol

Two types of blood samples were collected from calves under investigation. The first blood samples were collected on heparinized tube and the second was collected on plain tubes for obtaining clear sera. Complete blood picture were determined using VetScan HM5 Hematology system.

Serum enzymes including AST, ALT, and ALP were estimated according to the methods previously described by Kachmar and Moss (1987) [33], Bergmeyer and Harder (1986) [4] and Varley et al. (1980) [31] respectively. The levels of total serum protein, albumin, TAG, LDL-c, HDL-c and total cholesterol were determined according to the methods previously described by Doumas et al., (1981) [7]; Henry (1966) [20]; Fossati and Prencipe (1982) [15]; Friedwald et al. (1972) [17]; Demacker et al. (1980) [6] and Richmond (1973) [27], respectively. In addition, VLDL-c was calculated by division of TAG/5 mg dL<sup>-1</sup> [3].

Serum Alpha-1-acid glycoprotein (AGP) was estimated using a

commercial radial immune diffusion kit supplied by Ecos Institute (Furukawa, Miyagi, Japan). The procedure recommended by the manufacturer was monitored and the test outcome was read after 48 h incubation in a humid chamber at room temperature. The values of AGP were reported in mg/L.

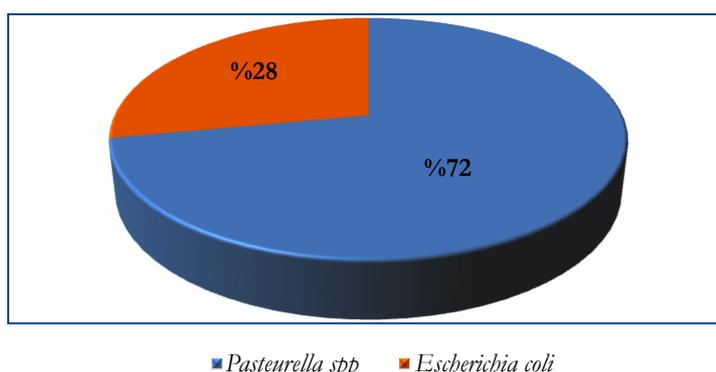
### Statistical analysis

All data was presented as mean ± standard error of mean by using student-t-test. All tests were performed using computer package of the statistical analysis system [28].

### Results

The laboratory results revealed a detected elevation in the values of leukocytic count and neutrophil percentage in calves with pneumonia when compared with control groups (Table 1). Moreover, there was a significant elevation in the levels of TAG, LDL-c, ALT, AST, ALP and AGP with significant decrease in the levels of total protein, albumin, cholesterol, HDL-c in pneumonic calves when compared with control calves (Table 2 and Figure. 2). The bacteriological examination of nasal swabs and bronchoalveolar lavage in calves under investigation revealed a presence of predominant two classes of microorganisms shared in induction of pneumonia in calves. These microorganisms were *Pasteurella* spp., (72%), and *Escherichia coli* (28%) as shown in Figure 1.

**Figure 1. The percentatge of isolated bacteria in cases of pneumonia in calves.**



**Table 1. Hematological parameters in control and diseased calves.**

| Variable                                   | Healthy calves | Pneumonic Calves |
|--|----------------|------------------|
| RBCs (x 10 <sup>6</sup> /mm <sup>3</sup> ) | 9.56 ± 0.52    | 8.23 ± 0.22*     |
| PCV %                                      | 26.32 ± 1.25   | 27.11 ± 1.24     |
| Hb g/dL                                    | 12.36 ± 1.45   | 10.45 ± 1.32*    |
| TLC (x 10 <sup>3</sup> /mm <sup>3</sup> )  | 9.56 ± 0.65    | 17.55 ± 2.32*    |
| MCV(f)                                     | 22.36 ± 1.45   | 37.22 ± 2.35*    |
| MCH (pg)                                   | 13.47 ± 0.68   | 9.32 ± 0.63*     |
| MCHC (%)                                   | 32.54 ± 1.25   | 27.31 ± 1.32*    |
| Neutrophil (%)                             | 44.25 ± 2.35   | 68.13 ± 3.34*    |
| Lymphocytes (%)                            | 48.25 ± 1.54   | 31.25 ± 2.45*    |
| Monocytes (%)                              | 1.24 ± 0.21    | 1.25 ± 0.14      |

\*Means are significantly different at the level (P ≤ 0.05).

RBCs, Red blood cells; PCV, Packed cell volume; Hb, hemoglobin; TLC, total leukocytic count; MCV, Mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

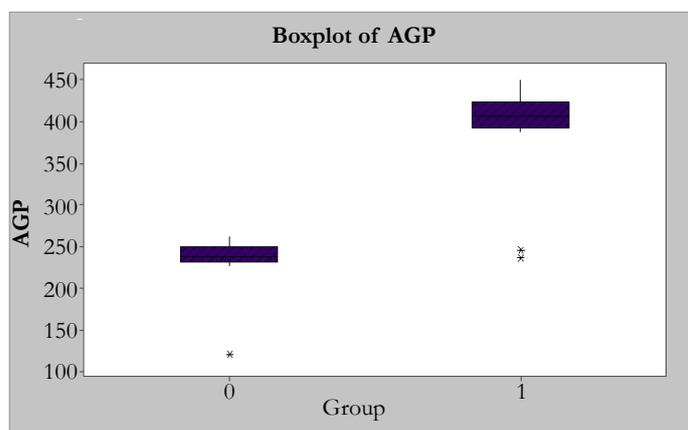
**Table 2. The biochemical blood picture of control calves and those with pneumonia.**

| Variable              | Healthy calves | Pneumonic Calves |
|-----------------------|----------------|------------------|
| AST (IU/l)            | 88.9 ± 4.73    | 141.33 ± 7.23*   |
| ALP (IU/l)            | 169.4 ± 3.37   | 288.7 ± 8.44*    |
| ALT (IU/l)            | 22.14 ± 0.43   | 109.11 ± 5.34*   |
| Total proteins (g/dl) | 6.92 ± 0.12    | 5.44 ± 0.22*     |
| Serum albumin (g/dl)  | 4.62 ± 0.12    | 3.42 ± 0.21*     |
| Serum globulin (g/dl) | 2.23 ± 0.12    | 1.94 ± 0.13*     |
| AGP (mg/l)            | 234.99 ± 5.94  | 389.6 ± 13.3*    |
| TAG (mg/dL)           | 29.45 ± 0.86   | 39.7 ± 0.64*     |
| Cholesterol (mg/dL)   | 61.24 ± 1.45   | 39.45 ± 0.63*    |
| LDL-c (mg/dL)         | 23.54 ± 0.84   | 17.87 ± 0.75*    |
| VLDL-c (mg/dL)        | 6.32 ± 0.43    | 7.41 ± 0.24*     |
| HDL-c (mg/dL)         | 27.4 ± 0.37    | 18.8 ± 0.19*     |

\*Means are significantly different at the level (P ≤ 0.05).

AST, Aspartate aminotransferase; ALP, Alkaline Phosphatase; ALT, Alanine Aminotransferase; AGP, Alpha-1-acid glycoprotein; TAG, Triglyceride; LDL-c, low-density lipoprotein cholesterol; VLDL-c, Very Low-Density Lipoprotein cholesterol; HDL-c, High Density Lipoprotein cholesterol

**Figure 2. Boxplot of AGP levels in control (0) and pneumonic calves (1) AGP.**



AGP, Alpha-1-acid glycoprotein

## Discussion

In this study, investigation of the utility of AGP and lipid profile in diagnosis of cases of BRD in calves were carried out.

The detected ( $P \leq 0.05$ ) higher levels of total leucocytic count and neutrophils percentage in pneumonic calves (Table 1) may be endorsed to a range of immunomodulatory effects [12]. Former investigations [5, 10] stated such elevation in total leucocytic count in calves with bacterial pneumonia. Furthermore, the elevated leucocytic count was recorded in many infectious diseases [22, 5]. From the other side, the detected elevation of AST, ALT, and ALP levels and decreased liver albumin synthesis in pneumonic calves (Table 2) may be allied with possible hepatic dysfunction persuaded by inflammatory response (pneumonia). Nikolic et al. (2006) [24] detected comparable higher values of ALT, AST and ALP in rats and Civelek et al., (2007) [5] in neonatal calves.

The consequence of inflammatory reaction on hepatic biosynthesis of albumin still debatable [25]. Conversely, decreased albumin values observed in this investigation comes in concurrence with earlier findings stated by Civelek et al., (2007). Comparable

significant decrease in the HDL-c and total cholesterol values, accompanied by significant higher levels ( $P \leq 0.05$ ) of VLDL-c and triglycerides of pneumonic calves were formerly detected in patients with septic infection [2, 16] and pneumonic buffalo-calves [10]. The decreased values of serum cholesterol in pneumonic calves may be ascribed to subsequent changes in either lipoprotein metabolism or liver dysfunction or inflammatory processes [5]. Decreased values of HDL-c possibly ascribed to its protective effects against inflammation which intermediated via bacterial endotoxins binding and subsequent neutralization [32]. Moreover, it was stated that inflammation leads to hypertriglyceridemia in both animals and human [1, 26]. This may be attributed to an increased synthesis of VLDL-c, diminished conversion of VLDL-c to LDL-c by the embarrassment of lipoprotein lipase action [19] or stimulation of hepatic and adipose tissue lipolysis as well as hepatic fatty acid synthesis, which serve as substrates for hepatic VLDL synthesis [13].

The primary way leading to significant increase in APP in infected calves in this study, may involve initial secretion of inflammatory cytokines by macrophages at the site of inflammation or infection. This results in a cascade of additional secretion of cytokines by macrophages and other immune cells. The most important stimulator of APPs are cytokines particularly, IL-1, IL-6

families and TNF [18].

Bacterial invasion stimulate a potent acute phase reaction inside the animal body whereas viral infections generally lead to weak or non-detectable acute phase reactions [8].

Inflammation leads to secretion of inflammatory cytokines like IL-1, IL-6 and tumor necrosis factor (TNF) which change the blood levels of a multiplicity of proteins that are created mostly in the hepatic cells [10]. The levels of these proteins is usually varied from low to non-detectable in healthy animals and elevated levels are used to diagnose and monitor animal diseases [18, 11]. The precise type of APPs and the time course for changes in these proteins differ by species based on the starting signal or causal inflammatory process [14].

The exact role of Alpha-1-acid glycoprotein (AGP) is not yet clear, however it binds to a variety of metabolites like histamine, heparin, and serotonin, catecholamine and steroids [21]. Moreover, it was reported that, AGP binds to pharmacological compounds, which may have therapeutic consequences as the amount bound can influence the metabolically active fraction of the drug. Elevated serum levels of AGP because of acute phase reaction may be due to its effect in reducing the levels of free drugs, thus influencing their pharmacokinetics.

Elevated levels of AGP in pneumonic calves in this study may be due to the involvement of AGP in plasma transport protein and immunomodulation of the inflammatory reply. Moreover, AGP may further defend the calves against invading bacteria, and acts as chaperone [29].

The binding and delivery prosperity of AGP is notable; assumed the contextual, that AGP could distinctly elevate its values during acute phase response, thus becoming one of the most plentiful proteins in serum [29, 9].

AGP has been categorized in a subset of lipocalins, the so-called immunocalins, a subfamily of proteins that may additionally regulate the inflammatory response against invading pathogens [23].

From the present study, it could be concluded that lipoprotein profile and alpha-1-acid glycoprotein could be used as diagnostic markers for pneumonia in calves.

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