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THE USE OF SERUM HAPTOGLOBIN AS AN INDICATOR OF INFLAMMATION IN BUFFALOE CALVES SUFFERING FROM PNEUMONIA

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ABSTRACT

This study aimed to evaluate the clinical diagnostic value of haptoglobin as one of acute phase proteins in buffaloes calves suffering from pneumonia. Fifteen calves (3-6 months) were used in this study. The apparently healthy group included 10 buffaloes calves, served as control. The calves belonged to some private farms in Sharkia Governorate. Nasopharyngeal swabs were collected from pneumonic calves for bacteriological examination. Blood samples were collected also for determination of serum total proteins, albumin and Hp. The obtained results revealed a significant increase in Hp levels in diseased animals than healthy ones. Also, an increase in serum total proteins accompanied with a decrease of serum albumin in diseased animals was observed. From this study, it can be concluded that, haptoglobin may act as an early indicators of infection and inflammation in buffaloe calve diseases.

INTRODUCTION

The acute phase proteins (APP) are a group of blood proteins that change in concentration in animals subjected to external or internal challenges such as infection, inflammation, surgical trauma or stress. The APP are considered to be non-specific innate immune components involved in the restoration of homeostasis and the restraint of microbial growth before animals develop acquired immunity to a challenge. The circulating concentration of the APP are related to the severity of the disorder and the extent of tissue damage in the affected animal, quantification of their concentration can therefore provide diagnostic and prognostic information (*Murata et al., 2004*).(*Orro et-al 2011*)

The APP is glycoproteins synthesized mainly by hepatocytes upon stimulation by pro-inflammatory cytokines and released into the blood stream (*Horadagoda et al., 1999*). They include haptoglobin, C - reactive protein, serum amyloid A, caeruloplasmin and fibrinogen.

Haptoglobin (HP), an alpha-globulin constituent, binds free haemoglobin, which is toxic and pro-inflammatory in the plasma and reduces the oxidative damage associated with haemolysis (*Yang et al., 2003*). It is considered to be the main APP in cattle and has been reported to be a useful indicator of bovine bacterial infection (*Eckersall and Conner 1988*). In ruminants its circulating level is negligible in normal animals, but increases over 100-fold on immune stimulation (*Conner et al., 1989*). Many studies have indicated the significance of Hp as a clinically useful parameter for measuring the occurrence and severity of inflammatory responses in cattle with mastitis, enteritis, pneumonia and endometritis (*Katoh et al., 1999*).

So, the aim of this study to quantify serum haptoglobin concentration in beef calves with naturally acquired clinical respiratory tract disease, also, we investigate the ability of serum haptoglobin concentration to be a diagnostic tool of the disease.

MATERIAL AND METHODS

(I) Animals:

Fifteen beef calves (3-6 months) belonging to a private farm in Sharkia Governorate, were used in this study. The calves showed signs of pneumonia (fever 41°C, nasal discharge, cough, dyspnea and rapid respiration). Other ten apparently healthy calves showed no signs of illness and served as a control group.

(II) Samples and adopted methods:

- (1) **Nasopharyngeal swabs:** these were collected from calves showing respiratory signs for bacteriological examination. The collected samples were examined bacteriologically according to *Brown et al. (1981)*.
- (2) **Blood samples:** The blood samples were collected from each animal, through jugular vein puncture, without anticoagulant to obtain clear, non-haemolysed sera. Albumin and total serum protein levels were determined according to the methods reported by Kits of Bio-Med Diagnostics (Egypt). Serum haptoglobin level was determined by means of Sodium Dodecyl Sulphat-Polyacrylamide Gel Electrophoresis (SDS – PAGE) according to *Yoshino et al., (1992)*.

Statistical analysis of the obtained data were performed according to *Snedecor and Cochran (1984)*.

RESULTS AND DISCUSSION

The results of this study were tabulated in tables (1 and 2). The bacteriological examinations of nasopharyngeal swabs (table 1) revealed that *Pasteurella multocida*, *Mannheimia haemolytica* type A, *E. coli* and *Staphylococcus aureus* are the most causative agents at percentages 30%, 20%, 20% and 15 % respectively. These results were nearly similar to those previously recorded by *Roberson et al. (1994)*. Also, *Wittum et al., (1996)* reported that *Pasteurella Multocida* is the most important infective agent associated with the respiratory tract disease in beef calves. The respiratory tract disease is the most commonly observed and reported disease condition in beef calves. Although the disease has been associated with multiple etiologic agents, alone or in combination, *P. multocida* is considered to be the principle agent of concern (*Wikse 1985*).

Regarding the biochemical analysis of serum, the results are tabulated in table (2). The levels of Hp are very low in healthy calves (control group). These agreed with those previously reported by *Marimatsu et al., (1992)* who mention that normal cattle have Hp concentration less than 0.1g/litre threshold value above 0.4 g /litre, considers presence of bacterial infection.

Recently, *Gronlund et al., (2005)* reported that Hp is present in normal bovine sera at a level of 100 ug/ml or less, and its level increase as much as 10 fold in response to a number of bacterial infections, and its concentration below the detection limit was considered as good indicator of healthy animal.

In this study, the relation between serum Hp and observed clinical disease was determined (table 2). Where *Wittam et al., (1996)* reported that the infection with *P. haemolytica* induce an acute-phase response and resulting large increase in serum Hp concentration, this increase is indicative of pulmonary bacterial infection. Also, *Conner et al., (1998)* reported a rapid and large increase in serum Hp concentration in calves inoculated with *P. haemolytica*. Lastly, *Fagliari et al., (2003)* found significant increase in serum Hp concentration in calves with pneumonic pasteurellosis, compared with control calves. In this study, our data indicate that feedlot calves with clinical respiratory tract disease have a large and variable Hp response.

Concerning serum albumin and serum total proteins levels in this study, there was a significant drop in serum albumin, accompanied by significant increase in serum total protein in diseased animal. Similar findings were reported by *Thomas (2000)* who mentioned that lower albumin level concomitant with the higher total protein level was characteristic for inflammatory process.

Eckersall and Conner (1988) reported that the acute phase response leads to increase serum levels of a number of positive acute phase proteins and to a concomitant decrease in negative acute phase proteins such as albumin. Recently, *Amina and Selim (2007)* attributed this decrease in serum albumin to the bacterial toxins which many affect the hepatic parenchyma resulting in failure of liver to synthesize albumin.

CONCLUSION

The acute phase proteins (APP) were produced during the early inflammatory response; represent a potentially useful marker of bacterial infection and other inflammatory diseases. These values in conjunction with other common measures might prove to be more useful as a diagnostic tool for some diseases in animals.

Table (1): Results of bacteriological examination of nasopharyngeal swabs

No. of samples	Isolated bacteria					
	Single isolates (n = 40 %)			Mixed isolates (n = 60%)		
		No.	%		No.	%
15	<i>Pasteurella multocida</i>	2	33	<i>P. multocida</i> (2) + <i>Mannheimia haemolytica</i> type A (2)	4	44
	<i>Mannheimia haemolytica</i> type A.	2	33	<i>Staph. aureus</i> (2) + <i>E. coli</i> (1)	3	33
	<i>Staphylococcus aureus</i>	1	17			
	<i>E. coli</i>	1	17	<i>Streptococcus</i> species (1) + <i>E. coli</i> (1)	2	23

Table (2): Mean values serum total protein, albumin and haptoglobin in both apparently healthy and pneumonic calves

Parameters	Calves	
	Apparently healthy	Pneumonic calves
Total protein (gm/dl)	6.8 ± 0.21	7.81 ± 0.13**
Albumin (gm/dl)	3.1 ± 0.18	2.5 ± 0.52*
Haptoglobin (gm/dl)	0.221 ± 0.041	1.51 ± 0.19***

*Significant at (P < 0.05)

**Highly significant at (P < 0.01)

*** Very significant at (P < 0.001)

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المخلص العربي

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استهدفت هذه الدراسة معرفة مدى إمكانية استخدام Haptoglobin كإحدى دلالات الالتهاب للتعريف بمرض الالتهاب الرئوى فى العجول الجاموس، أجرى هذا البحث على عدد ١٥ عجل جاموس (٦٣ شهر) تعاني من الالتهاب الرئوى، وذلك على مجموعة ضابطة اشتملت على عدد ١٠ من أفراد العجول الأصحاء ظاهرياً. وذلك من بعض المزارع بمحافظة الشرقية. تم فى هذه الدراسة إجراء الفحوص البكتريولوجية لعزل المسببات المرضية لمرض الالتهاب الرئوى فى العجول. وكذلك اخذ عينات دم لقياس مستوى البروتين الكلى والألبيومين ومستوى Hp فى الحيوانات المريضة وكذلك المجموعة الضابطة.

تبين من خلال هذه الدراسة حدوث زيادة معنوية لمستوى Haptoglobin فى السيرم الحيوانات المريضة عن المجموعة الضابطة وذلك كرد فعل لحدوث الالتهاب - كما تبين حدوث نقص فى مستوى الألبيومين وزيادة فى مستوى البروتين الكلى فى الحيوانات المريضة عن المجموعة الضابطة.

نستخلص من هذه الدراسة أهمية قياس Haptoglobin كمؤشر لحدوث الالتهاب مما يجعلها ذات دلالة لتشخيص العدوى المريضة بجانب الطرق الأخرى المعروفة، وكذلك يمكن اعتبارها مؤشراً للحالة الصحية العامة للحيوان.