

THE USE OF INFLAMMATORY MARKERS FOR IDENTIFICATION OF SOME DISEASES IN BUFFALOES

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Abstract

This study aimed to evaluate the clinical diagnostic value of some acute phase proteins (Haptoglobin, C- reactive proteins, fibrinogen) as a markers of infection and inflammation in buffaloes affected with mastitis and with pneumonia. twenty buffaloes (4-6 years) suffering from acute mastitis and 20 buffalo-calves (3 – 6 months) suffered from pneumonia were used in this study. The apparently healthy groups included 10 buffaloes and 10 buffalo-calves, served as control. The animals belonged to some private farms in Sharkia Governorate. Milk samples were collected from mastitic buffaloes, and nasopharyngeal swabs were collected from pneumonic buffalo-calves for bacteriological examination. Blood samples were collected also for determination of serum total proteins, albumin, CRP, Hp and plasma Fb. The obtained results revealed a significant increase in Hp, CRP, and Fb 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, the inflammatory markers (Hp, CRP, Fb) may act as an early indicators of infection and inflammation in some diseases in buffaloes.

INTRODUCTION

The acute phase proteins (APP) are a group of blood proteins that change in their 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 component 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 is related to the severity of the disorder and the extent of tissue damage in the affected animal; therefore, quantification of their concentration can provide a diagnostic and a prognostic information. Serum APP show species – related difference on their response to stimuli, Haptoglobin, Fibrinogen, and C- reactive protein are major APP in ruminants, but not in other species. Haptoglobin, Fibrinogen, and C- reactive protein are positive APP; they are glyco- proteins synthesized mainly by hepatocytes upon stimulation by pro- inflammatory cytokines and released into the blood stream. Inflammation, infection or tissue injury will trigger

cytokine release by defence oriented cells, thereby, inducing APP synthesis (Murata *et al.*, 2004).

Haptoglobin (Hp) is an alpha – globulin constituent, binds free haemoglobin, which is released either by haemolysis or during erythrocyte turn- over. It is a major APP in ruminants, its circulating level is negligible in healthy animals, but increases over 100-fold on immune stimulation in response to infection and tissue injury. Many studies have indicated the significance of Hp as a clinically useful parameter for measuring the occurrence and severity of inflammatory responses in cattle infected with mastitis (Ohtsuka *et al.*, 2001), and also with pneumonia (Heegaard *et al.*, 2000).

Fibrinogen (Fb) is involved in homeostasis, providing a substrate for fibrin formation, and in tissue repair, providing a matrix for the migration of inflammatory - related cells. It is used in cattle as a reliable indicator of the presence of inflammation, bacterial infection or surgical trauma (Thomas, 2000).

C- reactive protein (CRP) has been demonstrated in ruminants. It plays important roles in protection against infection, clearance of damaged tissue, prevention of auto immunization and regulation of the inflammatory response (Mold *et al.*, 2002).

The aim of this work is to evaluate the clinical diagnostic value of acute phase proteins (Hp, Fb, CRP) as a marker of infection and inflammation in buffaloes affected with mastitis and pneumonia.

MATERIALS AND METHODS

I- Animals

Thirty dairy buffaloes (3-5 years), and other thirty buffalo-calves (3-6 months), belonging to some private farms in Sharkia Governorate, were used in this study.

A- Twenty dairy buffaloes showed systemic and local clinical signs of acute mastitis (Body temperature, rumen motility, appetite, udder swelling and abnormal milk secretion). The other ten buffaloes were apparently clinically healthy and served as a control group.

B- Twenty buffalo-calves showed signs of pneumonia (fever 41°C, nasal discharge, cough, dyspnea and rapid respiration). The other ten calves showed no signs of illness and served as a control group.

II- samples and adopted methods

1- Milk samples

Procedures for collection and diagnosis of milk samples were performed as described by Brown *et al.*, (1981). The samples were positive for clinical mastitis, based on any observable signs in the udder and/or milk and delivered for

bacteriological examination. California mastitis test was applied on milk samples of each quarter of udder of clinically healthy individual buffalo.

2- Nasopharyngeal swabs

These were collected from buffalo-calves showing respiratory signs for bacteriological examination. The collected samples were examined bacteriologically according to Brown *et al.* (1981).

3- Blood samples

Two blood samples were collected from each animal, through jugular vein puncture, the first one was with Heparin as anticoagulant for measuring plasma fibrinogen level. The second blood sample was without anticoagulant to obtain clear, non-haemolysed sera. Plasma fibrinogen level, serum C-reactive protein, Albumin and total serum protein levels were determined according to the methods reported by Kits of Biosystems S.A. (Spain) & 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 1,2,3 and 4. The bacteriological examinations of milk samples (Table 1), revealed that the main isolates were *Staphylococcus aureus* (30%), *E-coli* (25%), *Streptococcus agalactia* (20%), *Corynebacterium pyogenes* (15%), and *Pseudomonas aeruginosa* (10%). These findings were nearly similar to those previously mentioned by Seddek (1996). The bacteriological examinations of nasopharyngeal swabs (Table 2) 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).

Regarding the biochemical analysis of serum and plasma, the results are tabulated in Tables 3&4. The levels of APP (Hp, CRP, Fb) are very low in healthy dairy buffaloes and buffalo-calves (control group). These agreed with those previously reported in cattle by Marimatsu *et al.* (1992), who mentioned that normal cattle have Hp concentration less than 0.1g/litre, threshold value above 0.4g/litre, considers presence of bacterial infection. Hp is a haemoglobin binding protein and is considered the main APP in cattle, its value of 10mg/ml has been considered to be within the physiological limits.

Recently, Gronlund *et al.* (2005) reported that Hp is present in normal bovine sera at a level of 100µg/ml or less, and its level increases 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 udder quarters.

On the other hand, the mastitic dairy buffaloes and pneumonic calves, showed a significant increase in the concentration of Hp, CRP and fibrinogen. These results agreed with those recorded by Eckersall and Conner (1988) who stated that the acute phase proteins are good indicators of acute infection, and reported that Hp was a prominent acute phase reactant in cows with mastitis, where besides the clinical signs of the bacterial counts in milk, the level of Hp is an important marker for inflammation. Also, Wittum *et al.* (1996) mentioned that Hp is suitable for use as an inflammatory marker in mastitis, and reported mean serum Hp concentration of 69 mg/dl for 26 cows affected with clinical mastitis.

Regarding APP response to clinical respiratory tract disease, little informations are available. Wittum *et al.* (1996) reported mean serum Hp value of 67 mg/dl in 60 feedlot calves with clinical respiratory tract diseases. Lastly, Fagliari *et al.* (2003) found significant increase in serum Hp concentration in calves with pneumonic pasteurellosis, compared with control calves. The mean serum Hp concentration peaked at 100mg/dl; this increase is due to the inflammation and lung damage.

Regarding the serum CRP levels in diseased animals in this study, there was a significant increase in its levels in these animals than in the healthy ones. These results coincide with those reported by Lee *et al.* (2003), who recorded that CRP increases rapidly in the sera of most animals suffering from acute inflammation caused by infection or tissue injury, and may be effectively used to determine and control udder illness in cows, where its level was the highest during naturally occurring infections, such as mastitis and other tissue inflammation, and this level is a marker or tool for evaluating the health status of a herd.

Concerning the plasma fibrinogen levels in mastitic buffaloes and pneumonic calves in this study, there was a significant increase in their levels in diseased animals than in healthy ones. Similar results were obtained by Eckersall and Conner (1988), who found an increase in mean plasma fibrinogen in sick cows than in normal ones, and attributed this increase to inflammation and tissue destruction, and reported that plasma fibrinogen levels correlated with the severity of the disease, levels of 1.000mg per 100 ml or more reflected a poor to bad prognosis.

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 animals. 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 increased 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 may affect the hepatic parenchyma resulting in failure of liver to synthesize albumin.

CONCLUSION

The acute phase proteins (APP) produced during the early inflammatory response, represent a potentially useful systemic marker of bacterial infection and other inflammatory diseases. Their values in conjunction with other common measures might prove to be more useful. They provide a method of determining the true health status, and as a diagnostic tool for some diseases in animals. The application of these APP to veterinary diagnosis seems promising, but needs further investigations.

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Table 1. Results of bacteriological examination of milk from mastitic buffaloes.

No. of samples	Isolated Bacteria					
	Single isolates (n = 12,60%)			Mixed isolated (n = 8,40%)		
		No.	%		No.	%
20	<i>Staphylococcus aureus.</i>	4	35	<i>Staphylococcus aureus</i>	3	38
	<i>Streptococcus agalacita</i>	3	25	(2) + <i>Streptococcus agalacita</i> (1)		
	<i>Corynbacterium Pyogenes</i>	1	8	<i>Corynbacterium pyogenes</i> (2) + <i>E- coli</i> (3)	5	62
	<i>E- coli</i>	2	16			
	<i>Pseudomonas aeruginosa</i>	2	16			

Table 2. Results of bacteriological examination of nasopharyngeal swabs.

No. of samples	Isolated Bacteria					
	Single isolates (n = 7,35%)			Mixed isolated (n = 13,65%)		
		No.	%		No.	%
20	<i>Pasteurella multocida</i>	2	29	<i>P. multocida</i> (4) + <i>Mannheimia haemolytica</i> type A (2)	6	46
	<i>Mannheimia haemolytica</i> type A	2	29	<i>Staphylococcus aureus</i> (2) + <i>E- coli</i> (1).	3	23
	<i>Staphylococcus aureus</i>	1	13			
	<i>E- coli</i>	2	29	<i>Streptococcus species</i> (3) + <i>E- coli</i> (1)	4	31

Table 3. Mean values of serum total protein, albumin, haptoglobin, C-reactive protein, and plasma fibrinogen in both apparently healthy and mastitic buffaloes.

<i>Parameters</i>	<i>Buffalo</i>	
	<i>Apparently healthy</i>	<i>Mastitic</i>
Total protein (gm/dl)	7.45 ± 0.22	8.81 ± 0.51 ^x
Albumin (gm/dl)	3.2 ± 0.41	2.14 ± 0.13 ^x
Haptoglobin (gm/dl)	0.286 ± 0.069	1.24 ± 0.23 ^{xxx}
C- reactive protein (mg/L)	5.4 ± 0.85	14.6 ± 2.1 ^{xxx}
Plasma Fibrinogen (mg/dl)	455 ± 38	721 ± 82 ^{**}

Table 4. Mean values serum total protein, albumin, haptoglobin, C-reactive protein and plasma fibrinogen in both apparently healthy and pneumonic calves.

<i>Parameters</i>	<i>Buffalo-calves</i>	
	<i>Apparently healthy</i>	<i>Pneumonic calves</i>
Total protein (gm/dl)	7.03 ± 0.29	8.11 ± 0.13 ^{xx}
Albumin (gm/dl)	3.4 ± 0.16	2.3 ± 0.41 ^x
Haptoglobin (gm/dl)	0.241 ± 0.035	1.11 ± 0.16 ^{xxx}
C- reactive protein (mg/L)	4.21 ± 0.32	10.48 ± 1.0 ^{xxx}
Plasma Fibrinogen (mg/dl)	380 ± 25	641 ± 71 ^{**}

x Significant at (P<0.05)

xx Highly Significant at (P<0.01)

xxx very Significant at (P<0.001)

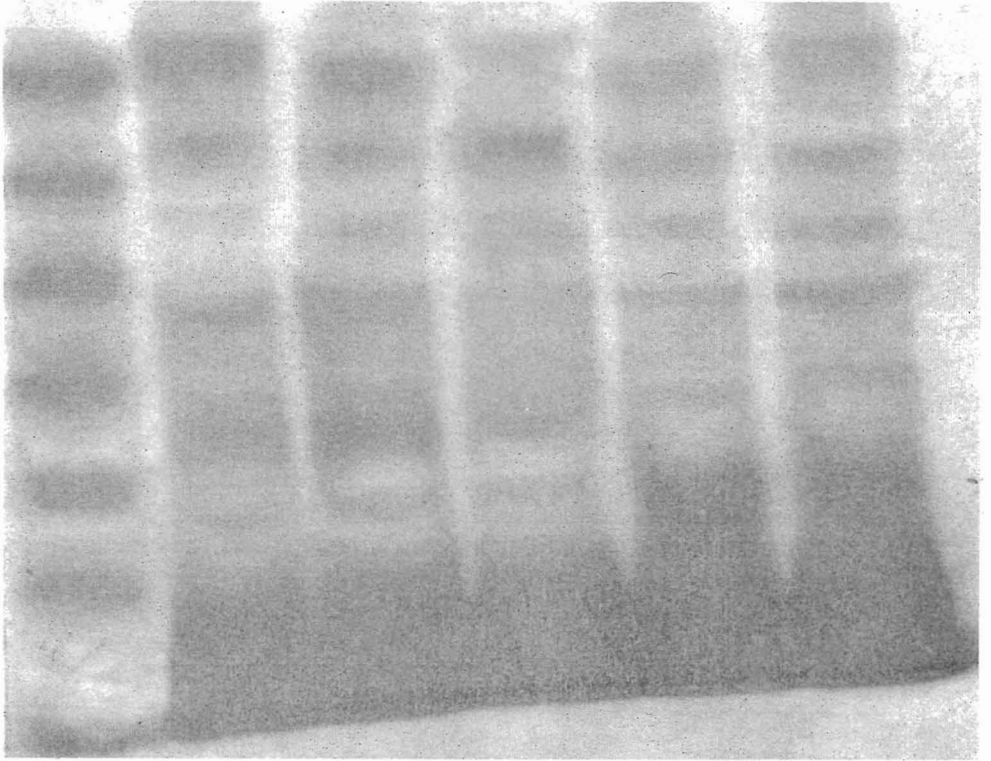
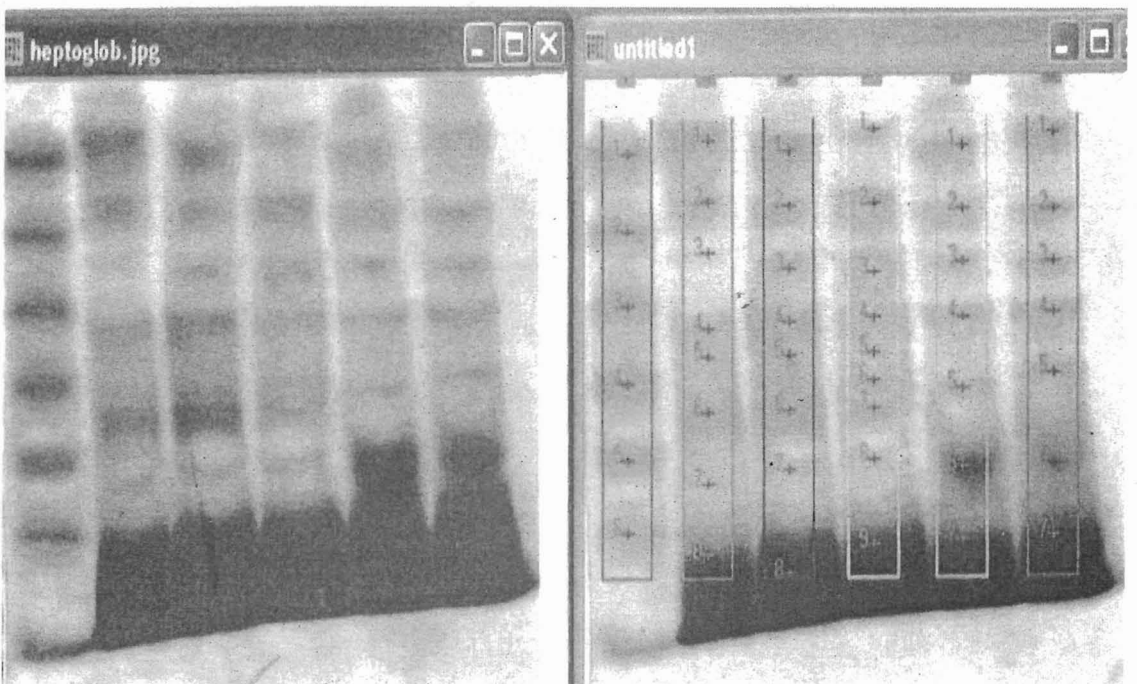


Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoretogram (SDS-PAGE).



The molecular weight (MW) of HP was estimated to be approximately 100.000K.D.

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إستخدام دلالات الإلتهاب للتعريف ببعض الأمراض في الجاموس

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استهدفت هذه الدراسة معرفة مدى إمكانية استخدام بعض دلالات الألتهاب (haptoglobin, C- reactive proteins, fibrinogen) والنتيجة عن حدوث العدوى المرضية للتعريف ببعض الأمراض مثل التهاب الضرع والالتهاب الرئوي في الجاموس. أجرى هذا البحث علي عدد ٢٠ جاموسة (٤-٦ سنوات) تعاني من التهاب الضرع الحاد وعدد ٢٠ عجل جاموسي (٣-٦ شهور) تعاني من الألتهاب الرئوي - وكذلك علي مجموعة ضابطة اشتملت علي عدد ١٠ من افراد الجاموس وعدد ١٠ من أفراد العجول الأصحاء ظاهرياً، وذلك من بعض المزارع الخاصة بمحافظة الشرقية. تم في هذه الدراسة إجراء الفحوص البكتريولوجية لعزل المسببات المرضية لمرض التهاب الضرع وكذلك مرض الالتهاب الرئوي في العجول. وكما تم أخذ عينات دم لقياس مستوى البروتين الكلي والألبومين ودلالات الألتهاب في الحيوانات المريضة وكذلك في المجموعة الضابطة. تبين من خلال نتائج هذه الدراسة حدوث زيادة معنوية لمستوى دلالات الألتهاب في السيرم وبلازما الحيوانات المريضة عن المجموعة الضابطة وذلك كرد فعل أولي لحدوث الألتهاب والعدوى المرضية بها - كما تبين حدوث نقص في مستوى الألبومين وزيادة في مستوى البروتين الكلي في الحيوانات المريضة عن المجموعة الضابطة.

نستخلص من هذه الدراسة أهمية قياس دلالات الألتهاب في بعض الحالات المرضية كونها مصاحبة لبداية حدوث العدوى المرضية والألتهاب - مما يجعلها ذات دلالة معينة لتشخيص تلك العدوى ومؤشراً للحالة الصحية العامة للحيوان.