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HAPTOGLOBIN AS A DIAGNOSTIC VALUE IN INFECTIOUS AND NON INFECTIOUS NEONATE DIARRHOEA IN BUFFALOE CALVES

(With 4 Tables and 2 Figures)

By

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القيمة التشخيصية للهبتوجلوبين في حالات الإسهال المسببة بعدوى والإسهال في عجول الجاموس حديثة الولادة

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تهدف هذه الدراسة إلى استخدام هابتوجلوبين الدم (بروتين حاد التفاعل) كمؤشر تشخيصي سريع في حالات الإسهال البكتيري والإسهال الناتج من الإضطرابات الهضمية حيث شملت الدراسة على عشرون عجل جاموسي منقسمين إلى مجموعتين الأولى تعاني من إسهال بكتيري والأخرى من إسهال نتيجة اضطراب في أسلوب الرضاعة بالإضافة إلى عشرة عجول سليمة كمجموعة ضابطة . كما أجريت بعض التحاليل البيوكيميائية وصور الدم وذلك لاستبيان التغيرات المصاحبة . وقد أوضحت النتائج زيادة معنوية في نسبة هابتوجلوبين الدم في العجول المصابة بالإسهال البكتيري وانخفاض معنوي في العجول المصابة بالإسهال الناتج من الإضطرابات الهضمية . كما وجدت زيادة معنوية في كل من العدد الكلي لكرات الدم البيضاء ونسبة خلايا النتروفيل ومستوى البروتين الكلي نتيجة زيادة جلوبيولين الدم في العجول المصابة بالإسهال البكتيري ، بينما وجد نقص معنوي فقط في مستوى البروتين الكلي نتيجة نقص الألبومين في العجول المصابة بالإسهال الناتج من الإضطرابات الهضمية.

SUMMARY

Blood serum haptoglobin concentration was measured using nor-partigen immuno-difusion plates. Some of the serum biochemical and haematological values in 20 diarrhoeic newlybron buffalo calves were also measured. Calves divided into 2 equal groups according to the

causative agent (bacterial diarrhoea and dietetic diarrhoa). A significant increase in the level of haptoglobin was obtained in the blood serum of calves suffering from bacterial diarrhoea, in contrast to a significant decrease in its value in serum of calves surffering from dietetic diarrhoea in comparison to apparently healthy calves. There was a significant positive correlation between haptoglobin and total leukocytic count, neutrophils and total serum proteins in calves suffering from bacterial diarrhoea while a negative relationship between haptoglobin and total serum proteins has been observed only in calves suffered fromdietetic diarrhoea.

Key words: *Haptoglobin, Neonate Diarrhoea.*

INTRODUCTION

Haptoglobin is one component of an acute phase protein, normally present in the blood serum of all domestic animals as well as humanbeings (Darlington et al., 1986 and Berkova et al., 1999). It is a glucoprotein component which precipitates with haemoglobin forming haemoglobin reactive protein (Spooner, 1970).

Haptoglobin is the third component of ∝2 globulins fraction of blood serum (Round, 1970). Although it has the physical characteristic of IgM antibody, it isn t immunoglobulin (Spooner, 1970). Each haptoglobin moleucule consists of two types of polypeptide chain ∝ and β wh ich linked by disulfide bands (Mc Murray, 1988).

Haptoglobin is produced by the hepatic cells in response to interleukin –1 (Bornstein, 1982), plays an important role in the recycling of iron in the circulation, thus preventing its excretion in the urine (Kent and Goodall, 1991) and to lessen the chance of microbial growth (Powanda, 1980). The liver is also the main site of haptoglobin destruction (Maury et al., 1988).

Haptoglobin has been reported to be a useful indicator in certain animal conditions, its values were increased in inflammatory processes, bacterial infections, trauma, biliary obstruction and burn (Spooner and Miller, 1971; Lokhorst and Breukink, 1975; Sheidrick et al., 1982; Whicher, 1984; Eckersall and Conner, 1988 and Skinner, 1994). Low haptoglobin levels are frequently associated with haemolytic and severe liver diseases (Silverman et al., 1986; Melicani et al., 1988 and Iman, 1990).

Haptoglobin concentration of more than 0.4 g/L indicate a significant infection (Skinner et al., 1991). A poor prognostic aspect was recorded in cattle and sheep with a serum haptoglobin concentration above 1.0 g/L (Echersall et al., 1988 and Scott et al., 1992).

The present investigation aimed to evaluate the role of haptoglobin as a diagnostic value in infectious and non infectious neonate diarrhoea in buffaloe calves.

MATERIALS and METHODS

I- Animals:

Twenty neonate diarrhoeic buffaloe calves of age up to 4 weeks were admitted to the Vet. Clinic. Fac. of Veterinary Medicine, Tanta University. Some of them were examined as ptaient in human being private farms. Calves were classified according to clinical signs nd causative agents into diarrhoea associated with bacterial infection and other due to dietetic errors. Also, 10 apparently healthy calves of the same ages were examined and collected samples of these animals were proved to be normal values and served as respective controls.

II) Blood samples:

Two blood samples were collected from jugular vein puncture of each calve, the first one into evacuated tubes containing EDTA for estimation of differentiated leukocytic count according to Schalm et al. (1975). The second blood samples was collected into evacuted tubes without anticlotting substances for estimation of serum haptoglobin, total proteins and albumin according to Barrett et al. (1979), Weichselbaum (1946) and Doumas et al. (1971) respectively. Serum haptoglobin is measured by using Nor-Partigen immuno-diffusion plates "Boehring" containing monospecific antiserum to the respective plasma protein stated on the label in a ready for use agarose-gel layer. Serum samples in volume of 0.5 µl were introduced into the wells of the plates and allowed to stand tightly closed at room temperature for 48 hours. A visible ring is formed when the antigen antibody reaction takes place and diameters were measured by using the Boehring Werke Measuring Viewer for immuno-analysis. The concentrations corresponding to the precipitate ring diameters measured are read from the table of calibration values appended (Table 1 and Figure 1).

III- Faecal samples:

Faecal samples were collected from all examined animals directly from the rectum by labelle sterilized plastic gloves and carried to

the laboratory on ice bags at once for parasitological examination (Monning, 1962) and for bacteriological examination (Quinn et al., 1994).

RESULTS

Animals of the first group explained severe yellow diarrhoea, fever and some signs of dehydration (mild-moderate sunken eye ball, mild-moderate loss of skin elasticity and loss of suckling affinity) and positive bacteriological examination for pathogenic *E.coli*. Animals of second group were characterized by milky or dark fetid diarrhoea, normal body temperature with negative pathogenic *E.coli* infection.

The results of haptoglobin in serum of diarrhoeic calves in both groups with respective controls as well as serum biochemical and haematological values in all groups were recorded in (Table 2 and Fig. 2).

DISCUSSION

There is little published information on the haptoglobin in newlyborn calves. Previous studies have been generally demonstrated in adult horse, sheep, cow and dogs. The mean serum haptoglobin in the healthy calves was 0.426 + 0.017 g/L (Table 2), while its value was significantly increase ($P<0.01$) in the serum of calves suggering. From bacterial diarrhoea. The results agree with the findings of Eckersall and Conner (1988), Skinner et al. (1991) and Maes et al. (1993), who demonstrated that serum haptoglobin vlaue was increased in bacterial infection and there is a significant positive correlation between the magnitude of the haptoglobin and severity of illness. Echersall et al. (1988) and Scott et al. (1992) found that diseased cattle and sheep with serum haptoglobin concentrations above 1.0 g/L represent a poor prognosis, so that accurate measurement is important.

A significant decrease ($P<0.01$) in serum haptoglobin of calves suffering from dietetic diarrhoea was obtained (Table 2). Low serum levels of haptoglobin are most frequently associated with diminished protein synthesis because it is synthesized in significant quantity only in the liver hepatocytes, stimulated by cytokines and other mediators (Silverman et al., 1986).

The results obtained for total leukocytic and neutrophils counts showed a positive correlation and signifiifant increased vlaue (932* & 631*) in correlation with serum haptoglobin of calves suffering from bacterial diarrhoea. Haptoglobin and neutrophils are parts of a highly interactive ensemble participating in inflammatory processes. Haptoglobin is taken up by neutrophils, stored within a cytoplasmic granular compartment and is secreted during phagocytosis by those cells (Berkova et al., 1999). Haptoglobin however may be enhanced locally at sites of inflammation to modulate granulocyte activity (Wagner et al., 1996), while serum haptoglobin in newlyborn calves suffering from dietetic error give insignificant correlation with total leukocytic ($r= 0.220$) and neutrophils (r= 0.304) counts (Table 4).

Significant increased total serum protein levels in calves suffering from bacterial diarrhoea were obtained. Table (2) showed significant increased in total globulin's due to an increased in α_2 globulins during the acute infection period (Sheidrick et al., 1982). Table (30 showed a positive signifiifant ($P<0.01$) correlation ($r=0.805^{**}$) between haptoglobin concentration and total proteins level in diarrhoeic calves suffering from bacterial diarrhoea. In contrast, a significant decreases in total serum protein due to decrease in serum albumin was obtained in dietetic diarrhoea and this could be related to anaroxic state of calves.

It could be concluded that haptoglobin can be performed quick, accurate, specific, sensitive and provide the clinician with a rapid screening test for differentiation between infectious and non infectious neonate diarrhoea in buffaloe calves.

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Table 1: Haptoglobin concentration by calibration values for nor-partigen immunodiffusions plates.

Diameter Mm	Hapt. g/l	Diameter mm	Hapt. g/l	Diameter mm	Hapt. g/l
4.0	0.380	4.6	0.772	5.2	1.220
4.1	0.442	4.7	0.843	5.3	1.300
4.2	0.505	4.8	0.915	5.4	1.380
4.3	0.569	4.9	0.989	5.5	1.460
4.4	0.635	4.5	1.060	5.6	1.550
4.5	0.703	5.1	1.140	5.7	1.630

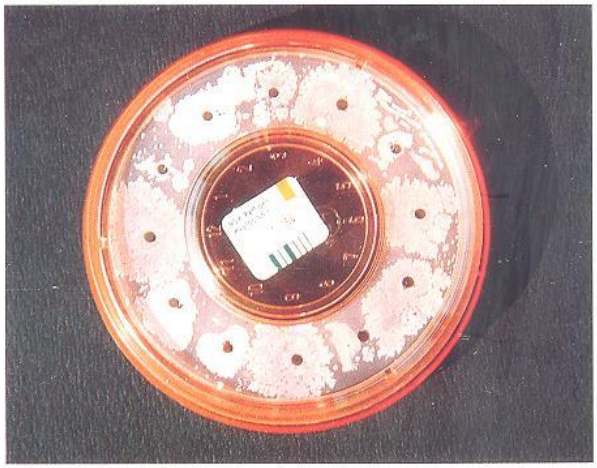

Figure 1: Examples of a Test for Haptoglobin (a) Bacterial diarrhoea (b) Dietetic diarrhoea.

Table (2)): Serum haptoglobin concentration and some serum-biochemical and haematological values in healthy and diseased calves .

Parameters	Unit	Healthy calves	Diseased Calves		ANOVA test
			Bacterial diarrhoea	Dietatic diarrhoea	
Haptoglobin	g/L	0.426± 0.017^a	0.639±0.012^b	0.345±0.010^c	**
Total proteins	g/L	0.71± 0.009^a	0.78±0.016^b	0.67±0.010^c	**
Albumin	g/L	0.44±0.003^a	0.41±0.009^b	0.37±0.002^c	**
Total globulins	g/L	0.31±0.001^a	0.39±0.003^b	0.32±0.004^a	**
A:G ratio	-	0.13±0.0001^a	0.10±0.0008^b	0.09±0.0007^c	**
T.WBCs	10^3/ul	13.7±0.584^a	16.04±0.551^b	13.67±0.598^a	**
Neutrophils	%	53.0±0.803^a	63.0±1.56^b	52.0±0.64^a	**

** Highly significant at P< 0.01

Figure (2): Serum haptoglobin concentration and some serum-biochemical and haematological values in healthy and diseased calves .

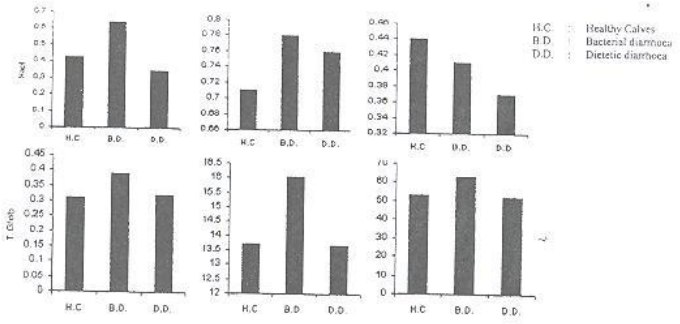

Table (3) : Matrix of simple correlation coefficient between the different paramrters in bacterial diarrhoea .

	Hapt.	T.protein	Albumin	T.glob	T.WBCs	Neut
Hapt.	1.000	0.805**	0.319	0.751*	0.932*	0.631*
T.protein		1.000	0.252	0.587	-0.155	0.390
Albumin			1.000	0.325	0.059	0.201
T.glob				1.000	0.150	0.211
T.WBCs					1.000	-0.001
Neut						1.000

Table (4) : Matrix of simple correlation coefficient between the different paramrters in dietetic diarrhoea .

	Hapt.	T.protein	Albumin	T.glob	T.WBCs	Neut
Hapt.	1.000	0.520**	0.073	0.025	0.220	0.304
T.protein		1.000	0.758*	-0.450	-0.590	-0.306
Albumin			1.000	0.675	-0.549	-0.129
T.glob				1.000	0.195	0.180
T.WBCs					1.000	-0.291
Neut						1.000