

INFLUENCE OF HYPOCUPROSIS ON THE IMMUNE RESPONSE OF SHEEP VACCINATED WITH INACTIVATED FOOT AND MOUTH DISEASE (FMD)VACCINE

By

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SUMMARY

A total of twenty one (21) balady sheep (1-3 years old) belonged to private flock at El- Banger area in Alexandria Governorate. Fourteen of these sheep were suffering from depraved appetite, unthriftiness, rough coat and pale mucous membrane. The rest seven sheep were clinically healthy used as a control group. Sheep under investigation were exposed to thoroughly clinical examination and parasitological analysis including fecal and blood examination, whereas, these animals were free from parasitism. All animals were vaccinated by FMD vaccine.

Biochemical results showed highly significant decrease in copper, iron and ceruloplasmin. Also, there were significant decrease of total protein, albumin and globulin. On the other hand, the haemogram result showed highly significant decrease in both haemoglobin and red blood cells, while there were significant decrease in the total leucocytic count. The differential leucocytic count results showed significant decrease in lymphocyte and significant increase of neutrophile. All these altered parameters retained nearly to normal within 4 weeks posttreatment..

The serological analysis showed highly significant decrease in the serum antibodies titre before and after four weeks of copper treatment.

It concluded that trace elements state especially copper play an important role in the immune response of vaccinated animals. Repair deficient serum copper before vaccination ensuer protective immunity level against diseases.

INTRODUCTION

The nutritional disorders cause great losses in the animal wealth; particularly copper deficiency, which impairs the animal ability to absorb mobilized iron from the decayed RBC's to reutilize it in haemoglobin synthesis. Copper plays an important role in normal bone formation and normal myelination of the brain cells and spinal cord as a component of the enzyme cytochrom oxidase, which is essential for myelin formation. Copper also known to play a vital role in production of healthy coat and wool with its crimp (Radostits, et al, 2000).

Copper is associated with ceruloplasmin in which copper transport protein that may be required in the incorporation of iron from the liver into transferrin for its transport to extrahepatic tissue, Kaneko (1990). Copper deficiency develops when the copper content of the ration was less than the animal requirements or when the copper in the ration is marginal to the normal but absorption and utilization of ingested copper was impaired by other minerals, Wikse et al; (1992).

Hypocuprosis major clinical signs in sheep are wool abnormalities with alopecia, emaciation, incoordination and bone abnormalities Church and Pond (1988) and Radosits (1994), Randhawa et al; (1997) and Saleh et al; (1998). There are many factors including nutritional deficiency as hypocuprosis that interfere with the immune response of vaccinated sheep. The control of foot and mouth disease depends mainly on vaccination of susceptible animals with specific vaccine that was found to be safe and immunogenic for goat and sheep in Egypt (Mobark, 1998).

Copper deficiency increases the susceptibility of lambs to microbial infections and selenium administration improved antibody responses, moreover cobalt deficiency reduces lambs survival and increases their susceptibility to parasitic infestation and impaired leucocyte and lymphocyte responses to invitro challenges (Suttle and Jones., 1989).

Hypocuprosis may cause immune dysfunction by changing the physiological characteristics that may be important in immunological state against pathogenic challenge as in viral infection, it changes the acute – phase protein response and affects lymphocyte responsiveness to mitogen stimulation, Stable et al; (1993) and Arthington et al; (1996).

Foot and Mouth disease (FMD) is one of the most fearful infectious disease of domestic livestock mainly cloven footed animals resulting in great economic losses (Radostits et al; 2000).

So, this study was aimed to evaluate the following some influences of hypocuprosis on immune response of sheep vaccinated with cell culture inactivated Foot and Mouth vaccine.

MATERIALS AND METHODS

1- Flock history:

The past history of this examined flock was poor growth rate, reduced the reproductive rate, lamb ataxia and failing of immune response that some vaccinated animal by FMD vaccine showed clinical signs of this disease even post vaccination. The present history revealed that most animals showed anophagia, diarrhea and depigmented steely wool.

2- Animals:

A total of twenty one (21) balady sheep (1-3 years old) belonged to private flock in El- banger area at Alexandria Governorate. Seven of them were clinically healthy used as a control group, while the fourteen sheep were suffering from depraved appetite, unthriftiness, rough coat with depigmented steely wool and pale mucous memberanes.

Sheep under investigation were exposed to full clinical examination and parasitological analysis including faecal and blood examination for procdud that the investigated sheep were free from parasitism (Kelly, 1984).

3- Vaccine and vaccination:

The examined animals were vaccinated by inactivated FMD vaccines that were purchased from Institute of Production and Research of Serum & Vaccines -Abasia - Egypt, and used as labeled by procedure (1ml s/c).

4-Blood samples, Haematological and Biochemical assay:

Two blood samples were collected from each animal by vein- puncture from jugular vein before and after treatment. The first one (3 ml volume) was collected in clean bottle containing 3 mg sodium ethylene diamine tetraacetic (EDTA) as anticogulant (Oser 1979) to determine haemoglobin (Hb) according to Crosby, et al; (1957), total red blood cell (RBCs), white blood cells (WBCs), according to Schalm, et al; (1975), and for preparing blood film, which stained by Giemsa stain to examine aganist blood parasites and differential leucocytic count.

The second sample (10ml) was collected in clean dry screw capped vails to obtain clear sera, these sera were analysed for quantitative determination of copper, iron, by using atomic absorbation spectrophotometer according to Bauer (1982) and Ramasy (1957) respectively. Ceruloplasmin was determined after Karl et al; (1974). Total protein and albumin were determined after the methods by Sonnenwirth and Jarette (1980) and Drupt (1974) respectively, while serum globulines were calculated by subtraction of the albumin values from the total protein. As well as antibodies titre against foot and mouth disease (FMD) were measured by serum neutralization test according to Ferreria (1976), the neutralizing index was calculated according to Karber et al; (1931) before and 4 weeks post- vaccination.

5- Diagnostic therapy and treatment:

Each one of affected sheep was orally administrated 1.5 gm copper sulphate in sufficient amount of water weekly for 3 successive weeks according to Radostits et al; (2000).

6- Statistical analysis:

The obtained results were statistically analysed using student "t" test after Selvin (1996).

RESULTS

1- The clinical observation:

Investigated sheep were exhibiting clinical sings of hypocuprosis as depraved appetite with pale mucous memberanes, emaciation, diarrhea, and alopecia, rough coat with depigmented steely appared wool. These animals were diagnosed after complete clinical examination "hypocuprosis" which were confirmed by biochemical analysis for the copper decreased levels.

All the previous clinical signs were subsides after oral copper therapy.

2- Biochemical analysis:

The alterations of serum copper, iron, ceruloplasmin, total protein, albumin and globulin were illustrated in Table (1) while Table (2) included the mean values of haemogram of copper deficient sheep before and after four weeks of treatments. On the other hand, Table (3) showed the mean values of antibodies titre of serum neutralizing in hypocuprosis suffering sheep vaccinated with inactivated Foot and Mouth disease vaccine (FMD).

DISCUSSION

The observed clinical signs on copper deficient sheep were ranged from depraved appetite, poor growth, alopecia rough coat, wool depigmentation, enlargement of joints and anaemia, such findings were previously recorded by Saleh et al; (1998). These clinical signs were confirmed by the concentrations of apparently healthy control. Also iron was highly significant decrease because copper is essential for iron metabolism therefore, the serum iron level decreased in copper deficient sheep, (Nabila 1983) and Madga and Hassan (1993).

The obtained results as shown in table (1) revealed that, there was a highly significant decrease in ceruloplasmin level in copper deficient group, ceruloplasmin oxidized Fe^{2+} to Fe^{3+} helping to transportation accross the intestinal wall (Blood and Radostits, 1989).

Serum total proteins, albumin and globulin were significantly decreased, the reduction in the total proteins may be attributed to the decrease in the serum albumin which due to lowering of synthetic power of albumin by the liver which caused due to under nutrition in deficient group, (Wyngarden and Smith, 1985).

The results of blood analysis were tabulated in table (2) in which there were highly significant decrease in both haemoglobin and red blood cells count which may be due to the decrease of iron, (Saleh et al; 1998). The total leucocytic count showed a significant decrease as well as the differential leucocytic count showed significant decrease in lymphocyte and significant increase of neutrophils which may be due to important of lymphocytes and neutrophil causing by copper deficiency, Suttle and Jones (1989) and Arthington et al; (1996).

The current results of immune response were showed in table (3) the titre serum neutralizing in copper deficient sheep vaccinated with (FMD) vaccine showed highly significant reducing during period of treatment and after 4 weeks of treatment when compared with the apparently healthy group and the copper deficient values didn't reach to the minimum protective level.

These result might be attributed to the reduced number of lymphocytes, which caused by hypocuprosis. Mobark, (1998) reported that copper deficient lambs vaccinated with inactivated FMD had significant low antibody index and not reach to the protective level.

The appearance of FMD outbreaks may be attributed to falling or breakdown of immunity due to stress factor rather than antigenic variations (Abou-Zaid and El-Nakashly., 1999) and hence hypocuprosis may represented one of these factors.

Stable et al; (1993), Rizkallah (1994) and Ceron et al; (1995) concluded that copper deficiency may affect various physiological characteristics that may be important in immunological defense against pathogen.

Through light of our results, we conclude that the monitoring of nutritional status especially for copper is important generally and specially before vaccination to give sufficient immune response to protect against pathogen and achieve a higher immune response after vaccination and avoid interference with the control programs.

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Table (1): Mean values of serum copper, iron, and ceruloplasmin, total proteins, albumin and (M±S.E) before and after four weeks of treatment.

Serum parameters	Before the treatment		After four weeks of treatment
	Control	cu def. Group	
Copper (µg/dL)	120.3±2.51	71.20±1.50*	115.2±3.21
Iron (µg/dL)	161.0±2.32	103.00±2.9**	157.0±2.75
Ceruloplasmine (IU/L)	115.0±3.11	64.20±2.10**	110.0±2.67
Total protein(gm/dL)	6.8±0.12	5.10±0.17*	6.4±1.09
Albumin (gm/dL)	3.6±0.13	2.25±1.06*	3.3±1.06
Globulin	3.2±0.02	2.85±0.07*	3.1±0.08

* Significant (P<0.01)

** Highly significant (p<0.001)

Table (2): Mean values of haemogram of copper deficient sheep before and after four weeks of treatment.

Blood parameters	Control groups	Hypocupramic sheep	
		Befor Treatment	After Treatment
Haemoglobin (gm/Dl)	12.12±0.30	8.21±0.52**	12.32±0.13
R.B.cs. (Mil/mm ³)	11.82±0.53	8.32±0.17**	12.00±0.09
W.B.cs. (thou/mm ³)	9.22±0.12	8.81±0.19*	8.97±0.32
Lymphocyte %	54.10±1.12	51.20±2.1	54.20±1.92
Monocyte	2.32±0.29	2.50±0.45	2.30±0.35
Neutrophil	40.00±2.1	43.77±0.17*	39.01±1.7
Eosinophil	2.58±0.37	2.53±0.14	2.49±0.95
Basophil	0	0	0

* Significant (P<0.01)

** Highly significant (P<0.001)

Table (3): Mean values of serum neutralizing antibodies titre in hypocuprosis suffering sheep vaccinated with inactivated Foot and Mouth Disease vacine (FMD).

Serum antibodies titer	During treatment		After 4 weeks of treatment Vaccinated	
	Control healthy (n = 7)	def.Group (n = 14)	Vaccinated Control group	vaccinated treatment group
FMD	1.26±0.03	0.55±0.08**	1.29±0.12	0.81±0.11**

The protective level for FMD is 1 log 10 TCID₅₀.

** P < (0.001).

المخلص العربي

تأثير نقص النحاس على الاستجابة المناعية في الأغنام المحصنة بلقاح مرض الحمى القلاعية المبيت

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تم الكشف الأكلينيكي على قطيع خاص من الأغنام البلدية بمنطقة البنجر بالإسكندرية وكان يعانى من سوء في الإنتاج، ضعف في الحالة التناسلية و نمو غير طبيعي مع خشونة في الصوف. تم اختيار ٢١ من الأغنام تتراوح أعمارها من ١-٣ سنوات. أربعة عشر منهم أظهرت الأعراض الآتية: انحراف الشهية، خشونة في الصوف مع شحوب في الأغشية المخاطية أما السبعة المتبقين فاستخدموا كمجموعة ضابطة. تم فحص جميع الحيوانات إكلينيكيًا و معمليًا للتأكد من خلوهم من الطفيليات الداخلية و الخارجية. تم تحصين القطيع بلقاح الحمى القلاعية و تطبيق التشخيص العلاجي على الحيوانات بتجربتهم بكريتات النحاس بجرعة ٥ جم أسبوعياً لمدة ٤ أسابيع. أخذت عينات دم من هذه الحيوانات قبل التحصين و قبل تجربتهم بكريتات النحاس وبعده بأربعة أسابيع متتالية لقياس مستوى المناعة و تأكيد التشخيص الإكلينيكي بأن هذا القطيع يعانى من نقص عنصر النحاس.

أظهرت نتائج التحليل البيوكيميائي نقص معنوي في عنصري النحاس و الحديد و نشاط أنزيم السريوبلزيم بمصل الدم. كما أوضحت نقص ملحوظ في البروتين الكلى ، الألبومين و الجلوبيولين الدم.

أظهرت نتائج تحليل الدم نقص شديد في عدد كرات الدم الحمراء و البيضاء خاصة الكرات الليمفاوية و المتعادلة و نسبة الهيموجلوبين الدم. كما أشارت نتائج التحليل السريولوجي إلى نقص ملحوظ في مستوى الأجسام المناعية لمرض الحمى القلاعية مقارنة بالمجموعة الضابطة. أظهرت النتائج تحسن ملحوظ في مستوى العناصر السابقة بعد العلاج فيما عدا مستوى الأجسام المناعية لمرض الحمى القلاعية وهذا يفسر أصابه القطيع بالحمى القلاعية بالرغم من التحصين المسبق.

على هذا نستج أن مستوى العناصر النادرة و خصوصاً عنصر النحاس يلعب دوراً أساسياً في الاستجابة المناعية للحيوانات المحصنة و يجب التوصية بضبط مستوى عنصر النحاس قبل التحصين لضمان مستوى مناعي وافي .