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## HAEMATOLOGICAL PICTURE AND SERUM PROTEIN ANALYSIS IN FATTENING BUFFALO CALVES NATURALLY INFESTED WITH THEILERIA ANNULATA

(With 2 Tables & 3 Fig.)

By

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### صورة الدم وتحليل سيرم البروتين في عجول التسمين الجاموسى المصابه طبيعيا بالثيليريا انيولاتا

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تم فحص عدد عشرة عجول تسمين مريضه بالمقارنه بأثنى عشر عجول تسمين سليمه . ظهر على العجول المريضه أعراض الحمى ، فقدان الشهيه ، بهتان الغشاء المخاطي ، اضطرابات تنفسيه ، وتضخم فى الغدد الليمفاويه السطحيه . بالاضافه إلى ذلك ظهر القراض بكميه كبيره على جسم العجول المريضه .

كانت نتيجة الفحص الميكروسكوبى لشريحة الدم المصبوغه بصيغة الجيمسا ايجابيه للثيليريا أنيولاتا قل عدد كرات الدم البيضاء وخاصة الخلايا الليمفاويه بينما ازاد عدد خلايا النيتروفيل ، خلايا الازينوفيل ، خلايا البازوفيل وخلايا المونوسيت فى العجول المريضه عنها فى العجول السليمه .

أظهر تحليل بروتين السيرم نقص معنوى فى البروتين الكلى ، زيادة غير معنويه فى الزلال ونقص معنوى فى الجلوبيولين فى العجول المريضه عنها فى الحيوانات السليمه .

كان هناك نقص فى الجلوبيولين فى العجول المريضه عنها فى السليمه . التشخيص المبكر للاصابه بالثيليريا مع تقديم غذاء غنى بالبروتين ورعايه جيده للحيوان يمكن أن يكون له قيمه جيده فى مكافحة الاصابه بالثيليريا .

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### SUMMARY

Diseased fattening buffalo calves (n=10) were examined in comparison with clinically healthy fattening ones (n=12). The diseased calves showed fever, anoxia, pale mucous membranes, some respiratory distress and enlargement of superficial lymph nodes. In addition ticks were found in large amount on the animal body. Stained blood films were positive for *Theileria annulata*. Leucopenia, lymphopenia with increased number of neutrophils, eosinophils, basophils and monocytes were found in diseased calves than healthy ones. Analysis of serum protein indicated significant decrease in total serum proteins, non-significant increase in serum albumin and significant decrease in serum globulins in diseased calves than healthy controls. The ratio of A/G was significantly higher in diseased calves than healthy control. Hypogammaglobulinemia was also observed in diseased calves.

### INTRODUCTION

Theileriosis is still a serious problem in cattle due to severe economic losses. Lowering vitality leads in addition to increased susceptibility of animals to bacterial and viral infection. FAHMY (1980) stated that *Theileria annulata* is considered the most important blood parasite among cattle and the infection rate was about 10% among cows and buffaloes in Assiut Province. SHERKOV (1976) SHARMA (1979); FAHMY (1980) and ABOUZID (1991) identified *Theileria annulata* parasites from naturally infected cattle by examining Geimsa's stained blood smears. OMUSE (1978); SHARMA (1979) and MOLLER (1981) reported lymphocytopenia in bovine theileriosis. ZIN-EL-ABDIN et al. (1976) mentioned that serum total proteins, serum albumin and A/G ratio decreased significantly in *Theileria annulata* infected cattle. MAXIE et al. (1982) and AbouZid (1991) found decreased total serum proteins in bovine theileriosis. The present investigation was designed to search for the possible changes in total and differential leucocytic count as well as changes in serum protein and protein fractionation accompanied the infestation with *Theileria annulata*.

### MATERIAL and METHODS

Ten diseased Fattening buffalo calves (12-16 months in age) from Kom-Abas. Assiut City. were examined in this investigation in comparison with other 12 clinically healthy ones used as control group.

The diseased calves showed fever. anorexia. pale mucous membranes, some respiratory distress and enlargement of superficial lymph nodes. In addition ticks were found in large amount on the animal body.

Blood smears from ear vein and two whole blood samples from jugular vein. One with EDTA and the other without EDTA were individually collected from infested and non-infested calves. Blood with EDTA was used for total leucocytic count and making blood film. Fixed blood films were prepared and stained by Giemsa stain for identification of blood parasites after MAHONEY and SALL (1961) and differential leucocytic count according to method of CART WRIGHT (1960). The whole blood samples without EDTA were used for separation of sera. Clear sera were analysed for total serum proteins. serum albumin. serum globulins and A/G ratio using test kits supplied from Biomerieux (France) and after the methods of WEICHSELBAUM (1946) and DOUMAS (1971). Electrophoresis of serum protein was conducted using the horizontal zone electrophoresis on agarose slides after JAIN (1986).

### RESULTS

Clinical examination revealed that calves suffered from signs of fever ( $40.3 - 41.2^{\circ}\text{C}$ ) anorexia loss of body weight, respiratory distress, polypnia. pale mucous membranes and presence of ticks on the body of animals. Some of diseased calves exhibited diarrhoea and enlargement of superficial lymph nodes, The mortality rate among calves was 8% (4 calves out of 50 calves) and the morbidity rate 20% (10 calves out of 50 calves).

Microscopical examination of stained blood smear showed intracellular *Theilaria annulata* infestation (Fig. 1).



Fig. (1): Blood film stained with Giemsa's stain showed intracellular *Theilaria annulata*.

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Blood examination for total and leucocytic differential count revealed leucopenia, basophilia, eosinophilia, neutrophilia and monocytosis and lymphopenia in diseased calves than healthy ones. The results are summarised in table 1.

Blood serum analysis for proteins indicated hypoproteinaemia and hyperalbuminaemia in diseased calves than healthy ones. Hypoglobulinaemia and increased A/G ratio was observed in diseased calves than healthy ones. The results are summarised in Table 2. Electrophoresis of serum protein indicated marked hypogammaglobulinaemia with slight changes in alpha-and beta globulins (Table 2 and Figs. 2 & 3).

Table 1: Mean Values of total and leucocytic differncial count in blood of fattening bull calves naturally infested with theileriosis and healthy control

Parameters	Units	Healthy Control calves	Diseased calves
TLC	( $10^3/\text{mm}^3$ )	8.23 $\pm$ 0.84** (6.5-9.4)	4.2 $\pm$ 0.63 (3.4 - 5.2)
Neutrophil Range	%	29.583 $\pm$ 3.729 (21 - 34)	40.700 $\pm$ 5.458** (33 - 52)
Band cell Range	%	0.917 $\pm$ 0.669 (0 - 2)	4.200 $\pm$ 1.398** (2 - 7)
Eosinophil Range	%	3.500 $\pm$ 1.087 (2 - 5)	5.600 $\pm$ 0.843** (5 - 7)
Basophil Range	%	0.5 $\pm$ 0.52 (0 - 1)	1.600 $\pm$ 1.075** (1 - 4)
Lymphocyte Range	%	61.583 $\pm$ 3.397** (57 - 70)	43.800 $\pm$ 3.521 (39 - 50)
Monocyte Range	%	3.917 $\pm$ 0.996 (2 - 5)	5 $\pm$ 943** (4 - 7)

Table 2: Analysis of serum protein and electrophoresis in calves naturally infested with theileriosis and clinical healthy control

Animal	T.P. gm/100ml	Alb. gm/100ml	Glob. gm/100ml	A/G	Globulins %			
					$\alpha$	$\beta_1$	$\beta_2$	$\gamma$
Control healthy calves	7.685±0.276 (7.25-8.02)	4.103±0.270 (3.70-4.80)	3.582±0.208 (3.30-4.07)	1.145±0.186 (0.80-1.50)	4	11	18	9
Diseased calves	6.820±0.630** (5.60-7.60)	4.320±0.815 (3.20-5.70)	2.520±0.466** (1.90-3.20)	1.799±0.662** (1.01-3.00)	4.5	9.5	18	6

+ Standard Deviation.  
 \* Significant variation ( $P < 0.05$ ).  
 \*\* Highly significant variation ( $P < 0.01$ ).

T.P. = Total protein  
 Alb. = Albumin  
 Glob. = Globulin  
 A/G = Albumin and globulin ratio

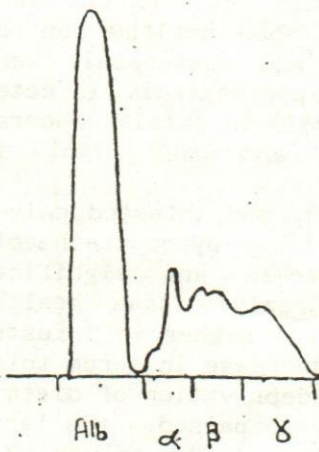


Fig. 2: Normal serum protein electrophoresis in fattening buffalo calves

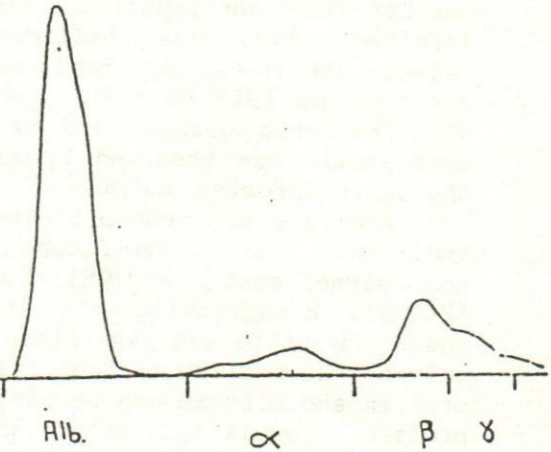


Fig. 3: Serum protein electrophoresis in fattening buffalo calves infested with theileria annulata.

## DISCUSSION

The clinical picture of diseased calves specially fever, enlargement of superficial lymph nodes, pale mucous membranes and presence of ticks on the animal body suggested theileria infestation. This was ascertained by presence of *Theileria annulata* in the stained blood films. HOOSHMAND-RAD (1976) reported that clinical observations in infested calves with a field strain of *Theileria annulata* and a gametogenous strain of *Theileria annulata* showed pyrexia and paler mucous membranes.

A significant variation in total leucocytic count was found between healthy and diseased calves, where leucopenia was markedly observed in calves infested with *Theileria annulata*. This was related to the severity of infestation with *Theileria annular*. Similar results were documented by SHARMA and GAUTANS (1971) who stated that theileria desintegrates the leucocytes resulting in leucopenia. OBI and ANOSA (1980) found extreme leucopenia in cattle with East coast fever. Differential leucocytic count of healthy and infested calves with *Theileria annulata* elicited marked lymphopenia, a fact that is coincided with Moller (1981), who observed that the decrease in leucocytic count is mainly in the lymphocyte fraction in cattle experimentally infested with *Theileria lawrenci*. Also there was monocytosis, eosinophilia, basophilia and neutrophilia in infested calves with *Theileria annulata* than healthy control calves. On the other hand neutropenia and eosinopenia were reported by LAIBLIN (1978) in steers experimentally infected with *Theileria annulata* and by MOLLER (1981) in cattle. Moderate monocytosis was observed by RAGHAVACHARI and RADDY (1959) in theileria infected sheep.

Analysis of serum proteins of healthy and infested calves indicated a significant ( $P < 0.01$ ) hypoproteinaemia non significant ( $P > 0.05$ ) hyperalbuminaemia and significant ( $P < 0.01$ ) hypoglobulinaemia in diseased calves than healthy ones. A/G ratio was significantly ( $P > 0.01$ ) higher in infested calves than healthy control calves. The decrease in serum total protein and albumin can be partly due to deprivation of dietary protein resulting from anorexia accompanied theileria infestation and/or poor nutritional status of the calves. ZIN EL-ABDIN et al. (1976) reported that total serum proteins, serum albumin and A/G ratio were decreased significantly in *Theileria annulata* infected cattle.

Electrophoresis of serum protein reflected slight changes in serum albumin, alpha-and beta globulins with marked decrease in gammaglobulin. The decrease in serum globulins, specially gammaglobulins means poor defensive mechanism in infested

calves. The decreased gamma globulin could result partly from the destructive effect of *Theileria annulata* on the site of formation of gamma globulin including lymphocytes, plasma cells, transitional cells of the lymph nodes, spleen and bone marrow. Such fact was proved by the obtained lymphopenia and anemia in the present infested calves. COLES (1980) stated that nutritional status of an animal has a marked effect on the synthesis of plasma proteins and lack of dietary protein has its marked effect on the levels of plasma gamma-globulins and albumin. The author added that a decrease in gamma globulins may result in impaired resistance to infectious agents. In addition, LAIBLIN (1978) reported that experimentally infected steers with *Theileria annulata* had aplastic anemia due to toxin-mediated lesions of the bone marrow.

#### REFERENCES

- Abouzeid, M.T. (1991): Clinical. Haematological and Immunological Studies On Parasitic Anaemia of Cattle. Thesis. Fac. Vet. Med. Assiut Univ.
- Cart Wright, G.G. (1960): Diagnostic Laboratory Haematology 4<sup>th</sup> Ed. Grune and Stratton. New York.
- Coles, E.H. (1980): Veterinary Clinical Pathology. 4<sup>th</sup> Ed. W. B. Saunders Company. Philadelphia and London.
- Doumas, B. (1971): Determination of serum albumin. Clin., Chem Acta. 31. P. 87.
- Fahmy, M. (1980): Parasitic Infection among calves in Egypt. Assiut Vet. Med. J., Vol. 7, No. 1: 21-23.
- Hooshmand-Rad, P. (1976): The pathogenesis of anaemia in *Theileria annulata* infection. Research in Veterinary Science 20 (3): 324-329.
- Laiblin, C. (1978): Clinical studies on *Theileria annulata* infection of cattle. 11. Haematological studies. Berliner and Munchener Tierarztliche Wochenschrift 91 (3): 48-50.
- Jain, N.C. (1986): Schalm's Veterinary Haematology 4<sup>th</sup> Ed. Les & Febiger. Philadelphia.
- Mahoney, D.F. and Sall, J.R. (1961): Bovine babesiasis: thick blood films for the detection of parasitemia. Aust. Vet. J. 37: P. 44.
- Maxie, M.G.; Dolan, T.T.; Jura, W.G.Z.; Tabel, H. and Flower, M.J. (1982): A comparative study of the diseases in cattle caused by *Theileria parva* or *T. lawrencei*, II. Haematology. clinical chemistry. coagulation studies and complement. Veterinary Parasitology. 10(1): 1-19.

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- Moller, M. (1981): Clinical and parasitological investigations on the course of experimental *Theileria lawrencei* infection in cattle. Inaugural Dissertation Frie Universtat. Berlin. P.97.
- Obi, T.U. and Anosa, V.O. (1980): Haematological studies on domestic animals in Nigeria. IV. Clinico-haemtological features of bovine trypanosomiasis. theileriosis. anaplasmosis. eperythrozosis & helminthiasis. Zentralblat fur Veterinar Medizin 27 B (9/10) 789-797.
- Omuse, H.J. (1978): A compartive haematological picture of field cases of East Coast Fever. anaplasmosis and badesiasis in bovines around Kabete. Edinburgh. UK: Center for Tropical Veterinary Medicine. 181-187.
- Raghavachari. K. and Raddy. A.M.K. (1959): Acute theileriasis in sheep. Indian. J. Vet. Sci-26. 123-124.
- Sharma. R.D. and Gautam. O.P. (1971): Theileriasis. II. Clinical cases in indigenous calves. Indian Vet. J. 48. 83-91.
- Sharma, N.N. (1979): Haematological observations in bovine theileriasis Anaplasmosis in mixed infection. Indian Journal of parasitology 3 (2): 153-155.
- Sherkov, N.; El-Rabie, Y. and Kokash, L. (1976): A survey of parasitic blood diseases "Tick-Borne fever" in Jordan Egypt. Vet. Sci., 13. (1): 29-35.
- Weichselbaum (1946): Determination of total protein. Am. J. Clin. Path., 16.P. 40.
- Zin-El-Abdin, Y.; Hamza, S.M. and El-Refaii, A.H. (1976): Some biochemical studies on "Imidocarb" in Friesian cattle infected with *Theileria annulata*. Egypt. J. Vet. 13 (2): 77-84.